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(54) Title: GENES AND POLYPEPTIDES RELATING TO HUMAN MYELOID LEUKEMIA

(57) Abstract: The present application provides novel human genes RHBDF1 whose expression is markedly elevated in CML and AML compared to normal peripheral blood cell, and lung adenocarcinoma compared to normal lung cell. The genes and polypeptides encoded by the genes can be used, for example, in the diagnosis of a cell proliferative disease, and as target molecules for developing drugs against the disease.

DESCRIPTION
GENES AND POLYPEPTIDES RELATING TO HUMAN MYELOID LEUKEMIA

The present application is related to USSN 60/414,867, filed September 30, 2002,
which is incorporated herein by reference.

Technical Field

The present invention relates to the field of biological science, more specifically to
the field of cancer research.   In particular, the present invention relates to novel genes,
RHBDF1, involved in the proliferation mechanism of cells, as well as polypeptides
encoded by the genes.   The genes and polypeptides of the present invention can be used,
for example, in the diagnosis of cell proliferative disease, and as target molecules for
developing drugs against the disease.

Background Art

Recent studies have demonstrated that information of gene expression profiles generated
by cDNA microarray analysis can provide very detailed nature of individual cancer cases
than traditional histopathological methods are able to supply.   The promise of such
information lies in its potential for improving clinical strategies for treating neoplastic
diseases and developing the novel drugs (Petricoin *et al.*, 2002. *Nat. Genet.*, 32 Suppl.,
474-479.).   Medical applications of microarray technologies include (i) discovery of
genes contributed to tumorigenesis, (ii) discovery of useful diagnostic biomarker(s) and
novel molecular target(s) for anti-cancer agents and (iii) identification of genes involved in
conferring chemosensitivity.   In fact, several potential clinical applications have started to
emerge as our understanding of these techniques.   Novel drugs targeting molecules that
have causative effects for cancer development have been proven to be very effective to
certain types of cancers.   For example, the ABL-selective tyrosine kinase inhibitor,
Imatinib methylate (Glivec; Novartis, Basel, Switzerland) dramatically improved the
management of chronic myeloid leukemia (CML) at the chronic phase (Druker *et al.*, 2001.
N. Engl. J. Med., 344, 1031-1037.).

To aim the above-mentioned goal, we also applied a microarray of human cDNA
consisting of 23,040 genes to analyze gene-expression profiles in tumors of various tissues
(Okabe *et al.*, 2001. *Cancer Res.*, 61, 2129-2137.; Kitahara *et al.*, 2002. *Neoplasia*, 4,
295-303.; Lin *et al.*, 2002.*Oncogene*, 21, 4120-4128.; Nagayama *et al.*, 2002. *Cancer Res.*,
62, 5859-5866.; Kaneta et al., 2002 Jpn. J. Cancer Res., 93, 849-856.; Okutsu *et al.*, 2002.
Mol. Cancer Ther., 1, 1035-1042.; Hasegawa *et al.*, 2002. *Cancer Res.*, 62, 7012-7017.;

Kikuchi *et al.*, 2003 *Oncogene*, 22, 2192-2205.).   Through analysis of these expression profiles, we have demonstrated that we identified *VANGL1* that was commonly up-regulated in HCCs, and that suppression of *VALGL1* expression by antisense oligonucleotides significantly decreased growth of HCC cells and induced apoptotic cell death (Yagyu *et al.*, 2002. Int. J. Oncol., 20, 1173-1178.).   Furthermore, using a genome-wide cDNA microarray, we have isolated several important genes involved in tumorigenesis such as *AF17* (Lin *et al.*,2001 *Cancer Res.* 61, 6345-6349.), *AXUD1* (Ishiguro *et al.*, 2001 Oncogene, 20, 5062-5066.), *HELAD1* (Ishiguro *et al.*, 2002 Oncogene, 21, 6387-6394.), *ENC1* (Fujita *et al.*, 2001. *Cancer Res.*, 61, 7722-7726.), *APCDD1* (Takahashi *et al.*, 2002. Cancer Res., 62, 5651-5656.), whose expression correlated to the activity of the transcription complex of T-cell factor/lymphoid enhancer–binding factor (Tcf-LEF) complex, and significantly elevated in colon-cancer cells.   The identification of these genes provides new opportunities for drugs aimed at targeting cancers.

Studies designed to reveal mechanisms of carcinogenesis have already facilitated identification of molecular targets for anti-tumor agents.   For example, inhibitors of farnexyltransferase (FTIs) which were originally developed to inhibit the growth-signaling pathway related to Ras, whose activation depends on posttranslational farnesylation, has been effective in treating Ras-dependent tumors in animal models (He et al., Cell 99:335-45, 1999).   Clinical trials on human using a combination or anti-cancer drugs and anti-HER2 monoclonal antibody, trastuzumab, have been conducted to antagonize the proto-oncogene receptor HER2/neu; and have been achieving improved clinical response and overall survival of breast-cancer patients (Lin et al., Cancer Res 61:6345-9, 2001).   A tyrosine kinase inhibitor, STI-571, which selectively inactivates bcr-abl fusion proteins, has been developed to treat chronic myelogenous leukemias wherein constitutive activation of bcr-abl tyrosine kinase plays a crucial role in the transformation of leukocytes.   Agents of these kinds are designed to suppress oncogenic activity of specific gene products (Fujita et al., Cancer Res 61:7722-6, 2001).   Therefore, gene products commonly up-regulated in cancerous cells may serve as potential targets for developing novel anti-cancer agents.

It has been demonstrated that CD8+ cytotoxic T lymphocytes (CTLs) recognize epitope peptides derived from tumor-associated antigens (TAAs) presented on MHC Class I molecule, and lyse tumor cells.   Since the discovery of MAGE family as the first example of TAAs, many other TAAs have been discovered using immunological approaches (Boon, Int J Cancer 54: 177-80, 1993; Boon and van der Bruggen, J Exp Med 183: 725-9, 1996; van der Bruggen et al., Science 254: 1643-7, 1991; Brichard et al., J Exp Med 178: 489-95, 1993; Kawakami et al., J Exp Med 180: 347-52, 1994).   Some of the

discovered TAAs are now in the stage of clinical development as targets of immunotherapy. TAAs discovered so far include MAGE (van der Bruggen et al., Science 254: 1643-7, 1991), gp100 (Kawakami et al., J Exp Med 180: 347-52, 1994), SART (Shichijo et al., J Exp Med 187: 277-88, 1998), and NY-ESO-1 (Chen et al., Proc Natl Acad Sci USA 94:

5   1914-8, 1997).   On the other hand, gene products which had been demonstrated to be specifically overexpressed in tumor cells, have been shown to be recognized as targets inducing cellular immune responses.   Such gene products include p53 (Umano et al., Brit J Cancer 84: 1052-7, 2001), HER2/neu (Tanaka et al., Brit J Cancer 84: 94-9, 2001), CEA (Nukaya et al., Int J Cancer 80: 92-7, 1999), and so on.

10   In spite of significant progress in basic and clinical research concerning TAAs (Rosenbeg et al., Nature Med 4: 321-7, 1998; Mukherji et al., Proc Natl Acad Sci USA 92: 8078-82, 1995; Hu et al., Cancer Res 56: 2479-83, 1996), only limited number of candidate TAAs for the treatment of adenocarcinomas, including colorectal cancer, are available.   TAAs abundantly expressed in cancer cells, and at the same time which

15   expression is restricted to cancer cells would be promising candidates as immunotherapeutic targets.   Further, identification of new TAAs inducing potent and specific antitumor immune responses is expected to encourage clinical use of peptide vaccination strategy in various types of cancer (Boon and can der Bruggen, J Exp Med 183: 725-9, 1996; van der Bruggen et al., Science 254: 1643-7, 1991; Brichard et al., J Exp

20   Med 178: 489-95, 1993; Kawakami et al.,J Exp Med 180: 347-52, 1994; Shichijo et al., J Exp Med 187: 277-88, 1998; Chen et al., Proc Natl Acad Sci USA 94: 1914-8, 1997; Harris, J Natl Cancer Inst 88: 1442-5, 1996; Butterfield et al., Cancer Res 59: 3134-42, 1999; Vissers et al., Cancer Res 59: 5554-9, 1999; van der Burg et al., J Immunol 156: 3308-14, 1996; Tanaka et al., Cancer Res 57: 4465-8, 1997; Fujie et al., Int J Cancer 80: 169-72,

25   1999; Kikuchi et al., Int J Cancer 81: 459-66, 1999; Oiso et al., Int J Cancer 81: 387-94 1999).

It has been repeatedly reported that peptide-stimulated peripheral blood mononuclear cells (PBMCs) from certain healthy donors produce significant levels of IFN-γ in response to the peptide, but rarely exert cytotoxicity against tumor cells in an

30   HLA-A24 or –A0201 restricted manner in ^{51}Cr-release assays (Kawano et al., Cance Res 60: 3550-8, 2000; Nishizaka et al., Cancer Res 60: 4830-7, 2000; Tamura et al., Jpn J Cancer Res 92: 762-7, 2001).   However, both of HLA-A24 and HLA-A0201 are one of the popular HLA alleles in Japanese, as well as Caucasian (Date et al., Tissue Antigens 47: 93-101, 1996; Kondo et al., J Immunol 155: 4307-12, 1995; Kubo et al., J Immunol 152:

35   3913-24, 1994; Imanishi et al., Proceeding of the eleventh International Hictocompatibility Workshop and Conference Oxford University Press, Oxford, 1065, 1992; Williams et al.,

Tissue Antigen 49: 129, 1997).   Thus, antigenic peptides of carcinomas presented by these HLAs may be especially useful for the treatment of carcinomas among Japanese and Caucasian.   Further, it is known that the induction of low-affinity CTL *in vitro* usually results from the use of peptide at a high concentration, generating a high level of specific

5   peptide/MHC complexes on antigen presenting cells (APCs), which will effectively activate these CTL (Alexander-Miller et al., Proc Natl Acad Sci USA 93: 4102-7, 1996).

Summary of the Invention

To comprehensively investigate the detailed molecular mechanism of

10   carcinogenesis, we have been attempting to obtain the genome-wide expression profiles of cancer cells from CMLs, acute myeloid leukemias (AMLs) and lung adenocarcinomas by means of cDNA microarray representing 23,040 transcripts (Kaneta et al., 2002,Jpn. J. Cancer Res., 93, 849-856.; Okutsu et al., 2002. Mol. Cancer Ther., 1, 1035-1042.; Kikuchi et al., 2003, *Oncogene*, 22, 2192-2205.).   Among the genes up-regulated in these cancers,

15   we identified the *RHBDF1* gene, similar to *Drosophila* Rhomboid-5, that is likely to belong to the Rhomboid family.   The Rhomboid family was isolated recently and their functions are indicated in only a limited number of organisms and contexts.   Among them, *Drosophila* Rhomboid-1 has been identified as an intramembrane serine protease that is responsible for initiating *Drosophila* epidermal growth factor receptor (EGFR) signaling

20   (Lee et al., 2001. Cell, 107, 161-171.; Urban et al., 2001. *Cell*, 107, 173-182.). Activation of this pathway in *Drosophila* is regulated by the selective proteolytic activation of the three transmembrane EGFR ligand precursors, Spitz, Keren and Gurken.   In their transmembrane forms, these ligands are inactive, being confined to the endoplasmic reticulum (ER).   In the signal-positive cell, Star, type2 membrane protein, exports these

25   ligands from the endoplasmic reticulum to the Golgi apparatus, where they are cleaved by rhomboid intramembrane serine proteases.   This cleavage releases the EGF ligand domains for subsequent secretion as active signals for other cells.   The protease active site of Rhomboids lies within the membrane bilayer, and the activating cleavage occurs within the ligand transmembrane domain. This proteolytic cleavage system is in contrast to other

30   known growth factors, which use cell surface metalloproteases to release the active growth factor domain   (Urban et al., 2002. Curr. Biol., 12, 1507-1512.).   Little is known about the function of nearly 100 currently known rhomboid-related genes that are conserved throughout evolution, but recent studies indicated that a Rhomboid from pathogenic bacterium was involved in the production of a quorum-sensing factor (Rather et al., 1994.

35   J. Bacteriol., 176, 5140-5144.; Gallio et al., 2000. Curr. Biol., 10, R693-694.), suggesting conservation of a Rhomboid-associated intercellular signaling mechanism during evolutional steps.

According to recent functional analysis of prokaryotic rhomboids as mentioned above, it has been understood that all Rhomboid proteins possess an intramembrane serine protease function.   For example, *Drosophila* Rhomboids 1-4 have similar proteolytic activities and all membrane-tethered ligands are substrates for the Rhomboid proteases (Lee *et al.*, 2001. Cell, 107, 161-171.; Urban *et al.*, 2002. *EMBO J.*, 21, 4277-4286.). However, although *RHBDF1* contained highly conserved rhomboid domain (Figure 1b and 1c), the essential residues for a serine protease that catalyze proteolysis were not conserved within this rhomboid domain.   Therefore, it would be of great interest to investigate whether *RHBDF1* protein might have proteolytic activity against membrane-tethered EGF receptor ligands such as Spitz.   Additional direct biochemical analysis of purified *RHBDF1* protein activity will be required to answer the above questions.

Our results strongly suggested the activated *RHBDF1* to function as oncogene on the basis of the facts that stable *RHBDF1* expression enhanced cell growth, and that reduction of *RHBDF1* expression by antisense S-oligonucleotide or RNAi suppressed growth of CML and lung-adenocarcinoma cells.   Furthermore, immunocytochemical staining indicated *RHBDF1* localized at Golgi apparatus like other Rhomboid proteins. These findings suggested that *RHBDF1* might have its own target substrates that mediate *RHBDF1*-dependent signaling, although such target molecules are currently unclear.   If so, identification of substrate for *RHBDF1* might provide us new clues to design novel anti-cancer drugs.

Thus, the present invention provides isolated novel gene, *RHBDF1* which is candidate as diagnostic marker for cancer as well as promising potential target for developing new strategies for diagnosis and effective anti-cancer agents.   Further, the present invention provides polypeptide encoded by this gene, as well as the production and the use of the same.   More specifically, the present invention provides the following:

The present application provides novel human polypeptide, *RHBDF1*, or a functional equivalent thereof, that promotes cell proliferation and is up-regulated in cell proliferative diseases, such as CML, AML and lung adenocarcinoma.

In a preferred embodiment, the RHBDF1 polypeptide includes a putative 855 amino acid protein with about 39% identity to Rhomboid-5 of *Drosophila melanogaster*. *RHBDF1* is encoded by the open reading frame of SEQ ID NO: 15 .   The SMART program predicted that RHBDF1 protein would contain a rhomboid domain consisting of the seven transmembrane domains at the C-terminal portion and suggested its Golgi location.   The *RHBDF1* polypeptide preferably includes the amino acid sequence set forth in SEQ ID NO: 16.   The present application also provides an isolated protein encoded from at least a portion of the *RHBDF1* polynucleotide sequence, or polynucleotide

sequences at least 40%, and more preferably at least 50% complementary to the sequence set forth in SEQ ID NO: 15.

The present invention further provides novel human gene, RHBDF1, whose expression is markedly elevated in a great majority of CML as compared to normal
5   peripheral blood cell.   The isolated RHBDF1 gene includes a polynucleotide sequence as described in SEQ ID NO: 15.   In particular, the RHBDF1 cDNA includes 2958 nucleotides that contain an open reading frame of 2568 nucleotides (SEQ ID NO: 15). The present invention further encompasses polynucleotides which hybridize to and which are at least 40%, and more preferably at least 50% complementary to the polynucleotide
10   sequence set forth in SEQ ID NO: 15, to the extent that they encode a RHBDF1 protein or a functional equivalent thereof.   Examples of such polynucleotides are degenerates and allelic mutants of SEQ ID NO: 15.

As used herein, an isolated gene is a polynucleotide the structure of which is not identical to that of any naturally occurring polynucleotide or to that of any fragment of a
15   naturally occurring genomic polynucleotide.   The term therefore includes, for example, (a) a DNA which has the sequence of part of a naturally occurring genomic DNA molecule in the genome of the organism in which it naturally occurs; (b) a polynucleotide incorporated into a vector or into the genomic DNA of a prokaryote or eukaryote in a manner such that the resulting molecule is not identical to any naturally occurring vector or
20   genomic DNA; (c) a separate molecule such as a cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), or a restriction fragment; and (d) a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a fusion polypeptide.

Accordingly, in one aspect, the invention provides an isolated polynucleotide that
25   encodes a polypeptide described herein or a fragment thereof.   Preferably, the isolated polypeptide includes a nucleotide sequence that is at least 60% identical to the nucleotide sequence shown in SEQ ID NO: 15.   More preferably, the isolated nucleic acid molecule is at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, identical to the nucleotide sequence shown in SEQ ID NO: 15.   In the case
30   of an isolated polynucleotide which is longer than or equivalent in length to the reference sequence, e.g., SEQ ID NO: 15, the comparison is made with the full length of the reference sequence.   Where the isolated polynucleotide is shorter than the reference sequence, e.g., shorter than SEQ ID NO: 15, the comparison is made to segment of the reference sequence of the same length (excluding any loop required by the homology
35   calculation).

The present invention also provides a method of producing a protein by transfecting

or transforming a host cell with a polynucleotide sequence encoding the *RHBDF1* protein, and expressing the polynucleotide sequence.   In addition, the present invention provides vectors comprising a nucleotide sequence encoding the *RHBDF1* protein, and host cells harboring a polynucleotide encoding the *RHBDF1* protein.   Such vectors and host cells

5   may be used for producing the *RHBDF1* protein.

An antibody that recognizes the *RHBDF1* protein is also provided by the present application.   In part, an antisense polynucleotide (e.g., antisense DNA), ribozyme, and siRNA (small interfering RNA or short interfering RNA) of the *RHBDF1* gene is also provided.

10   The present invention further provides a method for diagnosis of cell proliferative diseases that includes determining an expression level of the gene in biological sample of specimen, comparing the expression level of *RHBDF1* gene with that in normal sample, and defining a high expression level of the *RHBDF1* gene in the sample as having a cell proliferative disease such as cancer.   The disease diagnosed by the expression level of

15   *RHBDF1* is suitably a CML, AML or lung adenocarcinoma.

Further, a method of screening for a compound for treating a cell proliferative disease is provided.   The method includes contacting the *RHBDF1* polypeptide with test compounds, and selecting test compounds that bind to the *RHBDF1* polypeptide.

The present invention further provides a method of screening for a compound for

20   treating a cell proliferative disease, wherein the method includes contacting the *RHBDF1* polypeptide with a test compound, and selecting the test compound that suppresses the expression level or biological activity of the *RHBDF1* polypeptide.

The present application also provides a pharmaceutical composition for treating cell proliferative disease, such as cancer.   The pharmaceutical composition may be, for

25   example, an anti-cancer agent.   The pharmaceutical composition can be described as at least a portion of the antisense S-oligonucleotides or siRNAs of the *RHBDF1* polynucleotide sequence shown and described in SEQ ID NO: 15.   A suitable antisense S-oligonucleotide has the nucleotide sequence of SEQ ID NO: 11.   The antisense S-oligonucleotide of *RHBDF1* including those having the nucleotide sequence of SEQ ID

30   NO:11 may be suitably used to treat CML, AML or lung adenocarcinoma.   A suitable siRNA comprises a set of nucleotides with the nucleotide sequences of SEQ ID NOs: 13 and antisense sequence thereof as a target sequence.   The siRNA of *RHBDF1* consisting of nucleotide sequence of SEQ ID NO: 13 may be suitably used to treat CML, AML or lung adenocarcinoma.

35   The course of action of the pharmaceutical composition is desirably to inhibit growth of the cancerous cells.   The pharmaceutical composition may be applied to

mammals including humans and domesticated mammals.

The present invention further provides methods for treating a cell proliferative disease using the pharmaceutical composition provided by the present invention.

In addition, the present invention provides method for treating or preventing cancer,
5   which method comprises the step of administering the *RHBDF1* polypeptide.   It is expected that anti tumor immunity be induced by the administration of the *RHBDF1* polypeptide.   Thus, the present invention also provides method for inducing anti tumor immunity, which method comprises the step of administering the *RHBDF1* polypeptide, as well as pharmaceutical composition for treating or preventing cancer comprising the
10 *RHBDF1* polypeptide.

It is to be understood that both the foregoing summary of the invention and the following detailed description are of a preferred embodiment, and not restrictive of the invention or other alternate embodiments of the invention.

15 Brief Description of the Drawings

Figure 1   Expression profiling of CML using cDNA microarray analysis.

(a) Cy5/Cy3 signal intensity ratios of *RHBDF1* in 27 CML patients.

(b) A ClustalW-derived alignment of rhomboid domain in *Drosophila* Rhomboid-1 to Rhomboid-6 and C6135 (*RHBDF1*); The predicted positions
20   of the seven-transmenberane domains are indicated with blacklines.

(c) A phylogenetic tree derived from the ClustalW alignment of Rhomboid family

Figure 2   Characterization of the *RHBDF1* gene

(a) Northern blot of C6135 in various human tissues.   Molecular size is indicated at left side.
25 (b) Subcellular localization of Myc-tagged C6135 (*RHBDF1*) in NIH3T3 cells.

Figure 3   Effect of *RHBDF1* on growth of NIH3T3 cells

(a) Overexpression of exogenous transfected C6135 in NIH3T3.   Three cell lines are stably expressed compared to vector-transfectant.   Expression of glycerolaldehyde-3-phosphatase dehydrogenase (*GAPDH*) gene served as an
30   internal control.

(b) A growth rate of NIH3T3-C6135 cells and NIH3T3-vector cells.   The cells were cultured for 5 days followed by MTT assay to quantify the cell growth. This experiment was carried out in triplicate.   Bars indicate SD.

Figure 4 Growth-inhibitory effect of antisense S-oligonucleotides and small interfering
35 RNA (siRNA) designed to reduce expression of *RHBDF1* in K562 cells

(a) Expression of C6135 in K562 cells treated with either reverse (*RHBDF1*-R1) or

antisense (*RHBDF1*-AS1) S-oligonucleotides for 48h by semi-quantitative
RT-PCR.

(b) MTT assay using S-oligonucleotides (*RHBDF1*-R1 and *RHBDF1*-AS1) was
performed in K562 cells.   The values of untreated group were adjusted to 1.0.
This experiment was carried out five times.   Bars indicate SD.

(c) Expression of C6135 in K562 cells treated with either psiH1BX-*RHBDF1* or
psiH1BX-EGFP siRNA by semi-quantitative RT-PCR.

(d) MTT assay using siRNA (psiH1BX-*RHBDF1* and *psiH1BX-EGFP*) was
performed in K562 cells.   The values of untreated group were adjusted to 1.0.
This experiment was carried out five times.   Bars indicate SD.

Figure 5   Semi-quantitative RT-PCR analysis of C6135 expression

(a) in AML patients and (b) in lung adenocarcinoma cancer patients. Expression of
β-actin gene served as an internal control.   PB, peripheral blood; BM, bone
marrow.

Figure 6   Growth-inhibitory effect of antisense S-oligonucleotides and siRNA in lung
adenocarcinoma cells.

(a), (c) and (e), Semi-quantitative RT-PCR analysis of expression of *RHBDF1* in
A549, LC319, H522 cell lines, respectively, transfected with siRNA expression
vector.

(b), (d) and (f), colony formation assay were carried out in lung cancer cell line
A549, LC319 and H522, respectively.

(g) MTT assay was carried out in lung cancer cell line LC319, A549 and H522.This
experiment was carried out in triplicate.   Bars indicate SD.

Detailed Description of the Invention

The words "a", "an", and "the" as used herein mean "at least one" unless otherwise
specifically indicated.

The present application identifies novel human gene *RHBDF1* whose expression is
markedly elevated in CML compared to a normal peripheral blood cell.   The *RHBDF1*
cDNA consists of 2958 nucleotides that contain an open reading frame of 2568 nucleotides
as set forth in SEQ ID NO: 15.   The open reading frame encodes a putative 855-amino
acid protein.   The predicted amino acid sequence showed an identity of about 39% to
Rhomboid-5 of *Drosophila melanogaster*.   Therefore this protein was dubbed *RHBDF1*.

Consistently, exogenous expression of *RHBDF1* into cells conferred increased cell
growth, while suppression of its expression with antisense S-oligonucleotides or small
interfering RNA (siRNA) resulted in significant growth-inhibition of cancerous cells.

These findings suggest that *RHBDF1* render oncogenic activities to cancer cells, and that inhibition of the activity of these proteins could be a promising strategy for the treatment of cancer.

The present invention encompasses novel human gene *RHBDF1*, including a
5   polynucleotide sequence as described in SEQ ID NO: 15, as well as degenerates and mutants thereof, to the extent that they encode a *RHBDF1* protein, including the amino acid sequence set forth in SEQ ID NO: 16 or its functional equivalent.   Examples of polypeptides functionally equivalent to *RHBDF1* include, for example, homologous proteins of other organisms corresponding to the human *RHBDF1* protein, as well as
10   mutants of human *RHBDF1* proteins.

In the present invention, the term "functionally equivalent" means that the subject polypeptide has the activity to promote cell proliferation like *RHBDF1* protein and to confer oncogenic activity to cancer cells.   Whether the subject polypeptide has a cell proliferation activity or not can be judged by introducing the DNA encoding the subject
15   polypeptide into a cell expressing the respective polypeptide, and detecting promotion of proliferation of the cells or increase in colony forming activity.   Such cells include, for example, NIH3T3 cells, K562 cells, A549 cells, H522 cells, and LC319 cells.

Methods for preparing polypeptides functionally equivalent to a given protein are well known by a person skilled in the art and include known methods of introducing
20   mutations into the protein.   For example, one skilled in the art can prepare polypeptides functionally equivalent to the human *RHBDF1* protein by introducing an appropriate mutation in the amino acid sequence of either of these proteins by site-directed mutagenesis (Hashimoto-Gotoh et al., Gene 152:271-5, 1995; Zoller and Smith, Methods Enzymol 100: 468-500, 1983; Kramer et al., Nucleic Acids Res. 12:9441-9456, 1984;
25   Kramer and Fritz, Methods Enzymol 154: 350-67, 1987; Kunkel, Proc Natl Acad Sci USA 82: 488-92, 1985; Kunkel, Methods Enzymol 85: 2763-6 1988).   Amino acid mutations can occur in nature, too.   The polypeptide of the present invention includes those proteins having the amino acid sequences of the human *RHBDF1* protein in which one or more amino acids are mutated, provided the resulting mutated polypeptides are functionally
30   equivalent to the human *RHBDF1* protein.   The number of amino acids to be mutated in such a mutant is generally 10 amino acids or less, preferably 6 amino acids or less, and more preferably 3 amino acids or less.

Mutated or modified proteins, proteins having amino acid sequences modified by substituting, deleting, inserting, and/or adding one or more amino acid residues of a certain
35   amino acid sequence, have been known to retain the original biological activity (Mark et al., Proc Natl Acad Sci USA 81: 5662-6, 1984; Zoller and Smith, Nucleic Acids Res

10:6487-500, 1982; Dalbadie-McFarland et al., Proc Natl Acad Sci USA 79: 6409-13, 1982).

The amino acid residue to be mutated is preferably mutated into a different amino acid in which the properties of the amino acid side-chain are conserved (a process known as conservative amino acid substitution).   Examples of properties of amino acid side chains are hydrophobic amino acids (A, I, L, M, F, P, W, Y, V), hydrophilic amino acids (R, D, N, C, E, Q, G, H, K, S, T), and side chains having the following functional groups or characteristics in common: an aliphatic side-chain (G, A, V, L, I, P); a hydroxyl group containing side-chain (S, T, Y); a sulfur atom containing side-chain (C, M); a carboxylic acid and amide containing side-chain (D, N, E, Q); a base containing side-chain (R, K, H); and an aromatic containing side-chain (H, F, Y, W).   Note, the parenthetic letters indicate the one-letter codes of amino acids.

An example of a polypeptide to which one or more amino acids residues are added to the amino acid sequence of human *RHBDF1* protein is a fusion protein containing the human *RHBDF1* protein.   Fusion proteins are, fusions of the human *RHBDF1* protein and other peptides or proteins, and are included in the present invention.   Fusion proteins can be made by techniques well known to a person skilled in the art, such as by linking the DNA encoding the human *RHBDF1* protein of the invention with DNA encoding other peptides or proteins, so that the frames match, inserting the fusion DNA into an expression vector and expressing it in a host.   There is no restriction as to the peptides or proteins fused to the protein of the present invention.

Known peptides that can be used as peptides that are fused to the protein of the present invention include, for example, FLAG (Hopp et al., Biotechnology 6: 1204-10, 1988), 6xHis containing six His (histidine) residues, 10xHis, Influenza agglutinin (HA), human c-myc fragment, VSP-GP fragment, p18HIV fragment, T7-tag, HSV-tag, E-tag, SV40T antigen fragment, lck tag, α-tubulin fragment, B-tag, Protein C fragment, and the like.   Examples of proteins that may be fused to a protein of the invention include GST (glutathione-S-transferase), Influenza agglutinin (HA), immunoglobulin constant region, β-galactosidase, MBP (maltose-binding protein), and such.

Fusion proteins can be prepared by fusing commercially available DNA, encoding the fusion peptides or proteins discussed above, with the DNA encoding the polypeptide of the present invention and expressing the fused DNA prepared.

An alternative method known in the art to isolate functionally equivalent polypeptides is, for example, the method using a hybridization technique (Sambrook et al., Molecular Cloning 2nd ed. 9.47-9.58, Cold Spring Harbor Lab. Press. 1989).   One skilled in the art can readily isolate a DNA having high homology with a whole or part of the

DNA sequence encoding the human *RHBDF1* protein (i.e., SEQ ID NO: 15), and isolate
functionally equivalent polypeptides to the human *RHBDF1* protein from the isolated
DNA.   The polypeptides of the present invention include those that are encoded by DNA
that hybridize with a whole or part of the DNA sequence encoding the human *RHBDF1*

5   protein and are functionally equivalent to the human *RHBDF1* protein.   These
polypeptides include mammal homologues corresponding to the protein derived from
human (for example, a polypeptide encoded by a monkey, rat, rabbit and bovine gene).   In
isolating a cDNA highly homologous to the DNA encoding the human *RHBDF1* protein
from animals, it is particularly preferable to use tissues from trachea, thyroid, spinal cord,

10   prostate, skeletal muscle, or placenta.
       The condition of hybridization for isolating a DNA encoding a polypeptide
functionally equivalent to the human *RHBDF1* protein can be routinely selected by a
person skilled in the art.   For example, hybridization may be performed by conducting
prehybridization at 68°C for 30 min or longer using "Rapid-hyb buffer" (Amersham LIFE

15   SCIENCE), adding a labeled probe, and warming at 68°C for 1 hour or longer.   The
following washing step can be conducted, for example, in a low stringent condition.   A
low stringent condition is, for example, 42°C, 2X SSC, 0.1% SDS, or preferably 50°C, 2X
SSC, 0.1% SDS.   More preferably, high stringent conditions are used.   A high stringent
condition is, for example, washing 3 times in 2X SSC, 0.01% SDS at room temperature for

20   20 min, then washing 3 times in 1x SSC, 0.1% SDS at 37°C for 20 min, and washing twice
in 1x SSC, 0.1% SDS at 50°C for 20 min.   However, several factors, such as temperature
and salt concentration, can influence the stringency of hybridization and one skilled in the
art can suitably select the factors to achieve the requisite stringency.
       In place of hybridization, a gene amplification method, for example, the

25   polymerase chain reaction (PCR) method, can be utilized to isolate a DNA encoding a
polypeptide functionally equivalent to the human *RHBDF1* protein, using a primer
synthesized based on the sequence information of the protein encoding DNA (SEQ ID NO:
15).
       Polypeptides that are functionally equivalent to the human *RHBDF1* protein

30   encoded by the DNA isolated through the above hybridization techniques or gene
amplification techniques, normally have a high homology to the amino acid sequence of
the human *RHBDF1* protein.   "High homology" typically refers to a homology of 40% or
higher, preferably 60% or higher, more preferably 80% or higher, even more preferably
95% or higher.   The homology of a polypeptide can be determined by following the

35   algorithm in "Wilbur and Lipman, Proc Natl Acad Sci USA 80: 726-30 (1983)".
       A polypeptide of the present invention may have variations in amino acid sequence,

molecular weight, isoelectric point, the presence or absence of sugar chains, or form, depending on the cell or host used to produce it or the purification method utilized. Nevertheless, so long as it has a function equivalent to that of the human *RHBDF1* protein of the present invention, it is within the scope of the present invention.

5   The polypeptides of the present invention can be prepared as recombinant proteins or natural proteins, by methods well known to those skilled in the art. A recombinant protein can be prepared by inserting a DNA, which encodes the polypeptide of the present invention (for example, the DNA comprising the nucleotide sequence of SEQ ID NO: 15), into an appropriate expression vector, introducing the vector into an appropriate host cell,

10   obtaining the extract, and purifying the polypeptide by subjecting the extract to chromatography, for example, ion exchange chromatography, reverse phase chromatography, gel filtration, or affinity chromatography utilizing a column to which antibodies against the protein of the present invention is fixed, or by combining more than one of aforementioned columns.

15   Also when the polypeptide of the present invention is expressed within host cells (for example, animal cells and *E. coli*) as a fusion protein with glutathione-S-transferase protein or as a recombinant protein supplemented with multiple histidines, the expressed recombinant protein can be purified using a glutathione column or nickel column. Alternatively, when the polypeptide of the present invention is expressed as a protein

20   tagged with c-myc, multiple histidines, or FLAG, it can be detected and purified using antibodies to c-myc, His, or FLAG, respectively.

      After purifying the fusion protein, it is also possible to exclude regions other than the objective polypeptide by cutting with thrombin or factor-Xa as required.

      A natural protein can be isolated by methods known to a person skilled in the art,

25   for example, by contacting the affinity column, in which antibodies binding to the *RHBDF1* protein described below are bound, with the extract of tissues or cells expressing the polypeptide of the present invention. The antibodies can be polyclonal antibodies or monoclonal antibodies.

      The present invention also encompasses partial peptides of the polypeptide of the

30   present invention. The partial peptide has an amino acid sequence specific to the polypeptide of the present invention and consists of at least 7 amino acids, preferably 8 amino acids or more, and more preferably 9 amino acids or more. The partial peptide can be used, for example, for preparing antibodies against the polypeptide of the present invention, screening for a compound that binds to the polypeptide of the present invention,

35   and screening for accelerators or inhibitors of the polypeptide of the present invention.

      A partial peptide of the invention can be produced by genetic engineering, by

known methods of peptide synthesis, or by digesting the polypeptide of the invention with an appropriate peptidase. For peptide synthesis, for example, solid phase synthesis or liquid phase synthesis may be used.

Furthermore, the present invention provides polynucleotides encoding the
5   polypeptide of the present invention. The polynucleotides of the present invention can be used for the *in vivo* or *in vitro* production of the polypeptide of the present invention as described above, or can be applied to gene therapy for diseases attributed to genetic abnormality in the gene encoding the protein of the present invention. Any form of the polynucleotide of the present invention can be used so long as it encodes the polypeptide
10   of the present invention, including mRNA, RNA, cDNA, genomic DNA, chemically synthesized polynucleotides. The polynucleotide of the present invention includes a DNA comprising a given nucleotide sequences as well as its degenerate sequences, so long as the resulting DNA encodes a polypeptide of the present invention.

The polynucleotide of the present invention can be prepared by methods known to
15   a person skilled in the art. For example, the polynucleotide of the present invention can be prepared by: preparing a cDNA library from cells which express the polypeptide of the present invention, and conducting hybridization using a partial sequence of the DNA of the present invention (for example, SEQ ID NO: 15) as a probe. A cDNA library can be prepared, for example, by the method described in Sambrook et al., Molecular Cloning,
20   Cold Spring Harbor Laboratory Press (1989); alternatively, commercially available cDNA libraries may be used. A cDNA library can be also prepared by: extracting RNAs from cells expressing the polypeptide of the present invention, synthesizing oligo DNAs based on the sequence of the DNA of the present invention (for example, SEQ ID NO: 15), conducting PCR using the oligo DNAs as primers, and amplifying cDNAs encoding the
25   protein of the present invention.

In addition, by sequencing the nucleotides of the obtained cDNA, the translation region encoded by the cDNA can be routinely determined, and the amino acid sequence of the polypeptide of the present invention can be easily obtained. Moreover, by screening the genomic DNA library using the obtained cDNA or parts thereof as a probe, the
30   genomic DNA can be isolated.

More specifically, mRNAs may first be prepared from a cell, tissue, or organs (trachea, thyroid, spinal cord, prostate, skeletal muscle, or placenta) in which the object polypeptide of the invention is expressed. Known methods can be used to isolate mRNAs; for instance, total RNA may be prepared by guanidine ultracentrifugation
35   (Chirgwin et al., Biochemistry 18:5294-9, 1979) or AGPC method (Chomczynski and Sacchi, Anal Biochem 162:156-9, 1987). In addition, mRNA may be purified from total

RNA using mRNA Purification Kit (Pharmacia) and such or, alternatively, mRNA may be directly purified by QuickPrep mRNA Purification Kit (Pharmacia).

The obtained mRNA is used to synthesize cDNA using reverse transcriptase. cDNA may be synthesized using a commercially available kit, such as the AMV Reverse

5   Transcriptase First-strand cDNA Synthesis Kit (Seikagaku Kogyo).   Alternatively, cDNA may be synthesized and amplified following the 5'-RACE method (Frohman et al., Proc Natl Acad Sci USA 85: 8998-9002, 1988; Belyavsky et al., Nucleic Acids Res 17: 2919-32, 1989), which uses a primer and such, described herein, the 5'-Ampli FINDER RACE Kit (Clontech), and polymerase chain reaction (PCR).

10   A desired DNA fragment is prepared from the PCR products and ligated with a vector DNA.   The recombinant vectors are used to transform *E. coli* and such, and a desired recombinant vector is prepared from a selected colony.   The nucleotide sequence of the desired DNA can be verified by conventional methods, such as dideoxynucleotide chain termination.

15   The nucleotide sequence of a polynucleotide of the invention may be designed to be expressed more efficiently by taking into account the frequency of codon usage in the host to be used for expression (Grantham et al., Nucleic Acids Res 9: 43-74, 1981).   The sequence of the polynucleotide of the present invention may be altered by a commercially available kit or a conventional method.   For instance, the sequence may be altered by

20   digestion with restriction enzymes, insertion of a synthetic oligonucleotide or an appropriate polynucleotide fragment, addition of a linker, or insertion of the initiation codon (ATG) and/or the stop codon (TAA, TGA, or TAG).

Specifically, the polynucleotide of the present invention encompasses the DNA comprising the nucleotide sequence of SEQ ID NO: 15.

25   Furthermore, the present invention provides a polynucleotide that hybridizes under stringent conditions with a polynucleotide having a nucleotide sequence of SEQ ID NO: 15, and encodes a polypeptide functionally equivalent to the   *RHBDF1* protein of the invention described above.   One skilled in the art may appropriately choose stringent conditions.   For example, low stringent condition can be used.   More preferably, high

30   stringent condition can be used.   These conditions are the same as that described above. The hybridizing DNA above is preferably a cDNA or a chromosomal DNA.

The present invention also provides a vector into which a polynucleotide of the present invention is inserted.   A vector of the present invention is useful to keep a polynucleotide, especially a DNA, of the present invention in host cell, to express the

35   polypeptide of the present invention, or to administer the polynucleotide of the present invention for gene therapy.

When *E. coli* is a host cell and the vector is amplified and produced in a large amount in *E. coli* (e.g., JM109, DH5α, HB101, or XL1Blue), the vector should have "ori" to be amplified in *E. coli* and a marker gene for selecting transformed *E. coli* (e.g., a drug-resistance gene selected by a drug such as ampicillin, tetracycline, kanamycin,

5   chloramphenicol or the like).   For example, M13-series vectors, pUC-series vectors, pBR322, pBluescript, pCR-Script, etc. can be used.   In addition, pGEM-T, pDIRECT, and pT7 can also be used for subcloning and extracting cDNA as well as the vectors described above.   When a vector is used to produce the protein of the present invention, an expression vector is especially useful.   For example, an expression vector to be expressed

10   in *E. coli* should have the above characteristics to be amplified in *E. coli*.   When *E. coli*, such as JM109, DH5α, HB101, or XL1 Blue, are used as a host cell, the vector should have a promoter, for example, lacZ promoter (Ward et al., Nature 341: 544-6, 1989; FASEB J 6: 2422-7, 1992), araB promoter (Better et al., Science 240: 1041-3, 1988), or T7 promoter or the like, that can efficiently express the desired gene in *E. coli*.   In that respect,

15   pGEX-5X-1 (Pharmacia), "QIAexpress system" (Qiagen), pEGFP and pET (in this case, the host is preferably BL21 which expresses T7 RNA polymerase), for example, can be used instead of the above vectors.   Additionally, the vector may also contain a signal sequence for polypeptide secretion.   An exemplary signal sequence that directs the polypeptide to be secreted to the periplasm of the *E. coli* is the pelB signal sequence (Lei et

20   al., J Bacteriol 169: 4379, 1987).   Means for introducing of the vectors into the target host cells include, for example, the calcium chloride method, and the electroporation method.

In addition to *E. coli*, for example, expression vectors derived from mammals (for example, pcDNA3 (Invitrogen) and pEGF-BOS (Nucleic Acids Res 18(17): 5322, 1990), pEF, pCDM8), expression vectors derived from insect cells (for example, "Bac-to-BAC

25   baculovirus expression system" (GIBCO BRL), pBacPAK8), expression vectors derived from plants (e.g., pMH1, pMH2), expression vectors derived from animal viruses (e.g., pHSV, pMV, pAdexLcw), expression vectors derived from retroviruses (e.g., pZIpneo), expression vector derived from yeast (e.g., "Pichia Expression Kit" (Invitrogen), pNV11, SP-Q01), and expression vectors derived from *Bacillus subtilis* (e.g., pPL608, pKTH50)

30   can be used for producing the polypeptide of the present invention.

In order to express the vector in animal cells, such as CHO, COS, or NIH3T3 cells, the vector should have a promoter necessary for expression in such cells, for example, the SV40 promoter (Mulligan et al., Nature 277: 108, 1979), the MMLV-LTR promoter, the EF1α promoter (Mizushima et al., Nucleic Acids Res 18: 5322, 1990), the CMV promoter,

35   and the like, and preferably a marker gene for selecting transformants (for example, a drug resistance gene selected by a drug (e.g., neomycin, G418)).   Examples of known vectors

with these characteristics include, for example, pMAM, pDR2, pBK-RSV, pBK-CMV, pOPRSV, and pOP13.

In addition, methods may be used to express a gene stably and, at the same time, to amplify the copy number of the gene in cells.   For example, a vector comprising the

5   complementary DHFR gene (e.g., pCHO I) may be introduced into CHO cells in which the nucleic acid synthesizing pathway is deleted, and then amplified by methotrexate (MTX). Furthermore, in case of transient expression of a gene, the method wherein a vector comprising a replication origin of SV40 (pcD, etc.) is transformed into COS cells comprising the SV40 T antigen expressing gene on the chromosome can be used.

10   A polypeptide of the present invention obtained as above may be isolated from inside or outside (such as medium) of host cells, and purified as a substantially pure homogeneous polypeptide.   The term "substantially pure" as used herein in reference to a given polypeptide means that the polypeptide is substantially free from other biological macromolecules.   The substantially pure polypeptide is at least 75% (e.g., at least 80, 85,

15   95, or 99%) pure by dry weight.   Purity can be measured by any appropriate standard method, for example by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.   The method for polypeptide isolation and purification is not limited to any specific method; in fact, any standard method may be used.

For instance, column chromatography, filter, ultrafiltration, salt precipitation,

20   solvent precipitation, solvent extraction, distillation, immunoprecipitation, SDS-polyacrylamide gel electrophoresis, isoelectric point electrophoresis, dialysis, and recrystallization may be appropriately selected and combined to isolate and purify the polypeptide.

Examples of chromatography include, for example, affinity chromatography,

25   ion-exchange chromatography, hydrophobic chromatography, gel filtration, reverse phase chromatography, adsorption chromatography, and such (Strategies for Protein Purification and Characterization: A Laboratory Course Manual. Ed. Daniel R. Marshak et al., Cold Spring Harbor Laboratory Press, 1996).   These chromatographies may be performed by liquid chromatography, such as HPLC and FPLC.   Thus, the present invention provides

30   for highly purified polypeptides prepared by the above methods.

A polypeptide of the present invention may be optionally modified or partially deleted by treating it with an appropriate protein modification enzyme before or after purification.   Useful protein modification enzymes include, but are not limited to, trypsin, chymotrypsin, lysylendopeptidase, protein kinase, glucosidase, and so on.

35   The present invention provides an antibody that binds to the polypeptide of the invention.   The antibody of the invention can be used in any form, such as monoclonal or

polyclonal antibodies, and includes antiserum obtained by immunizing an animal such as a rabbit with the polypeptide of the invention, all classes of polyclonal and monoclonal antibodies, human antibodies, and humanized antibodies produced by genetic recombination.

5   A polypeptide of the invention used as an antigen to obtain an antibody may be derived from any animal species, but preferably is derived from a mammal such as a human, mouse, or rat, more preferably from a human. A human-derived polypeptide may be obtained from the nucleotide or amino acid sequences disclosed herein.

        According to the present invention, the polypeptide to be used as an immunization 10 antigen may be a complete protein or a partial peptide of the protein. A partial peptide may comprise, for example, the amino (N)-terminal or carboxy (C)-terminal fragment of a polypeptide of the present invention. A gene encoding a polypeptide of the invention or its fragment may be inserted into a known expression vector, which is then used to transform a host cell as described herein. The desired polypeptide or its fragment may be recovered 15 from the outside or inside of host cells by any standard method, and may subsequently be used as an antigen. Alternatively, whole cells expressing the polypeptide or their lysates, or a chemically synthesized polypeptide may be used as the antigen.

        Any mammalian animal may be immunized with the antigen, but preferably the compatibility with parental cells used for cell fusion is taken into account. In general, 20 animals of Rodentia, Lagomorpha, or Primates are used. Animals of Rodentia include, for example, mouse, rat, and hamster. Animals of Lagomorpha include, for example, rabbit. Animals of Primates include, for example, a monkey of Catarrhini (old world monkey) such as *Macaca fascicularis*, rhesus monkey, sacred baboon, and chimpanzees.

        Methods for immunizing animals with antigens are known in the art. 25 Intraperitoneal injection or subcutaneous injection of antigens is a standard method for immunization of mammals. More specifically, antigens may be diluted and suspended in an appropriate amount of phosphate buffered saline (PBS), physiological saline, etc. If desired, the antigen suspension may be mixed with an appropriate amount of a standard adjuvant, such as Freund's complete adjuvant, made into emulsion, and then administered 30 to mammalian animals. Preferably, it is followed by several administrations of antigen mixed with an appropriately amount of Freund's incomplete adjuvant every 4 to 21 days. An appropriate carrier may also be used for immunization. After immunization as above, serum is examined by a standard method for an increase in the amount of desired antibodies.

35   Polyclonal antibodies against the polypeptides of the present invention may be prepared by collecting blood from the immunized mammal examined for the increase of

desired antibodies in the serum, and by separating serum from the blood by any conventional method. Polyclonal antibodies include serum containing the polyclonal antibodies, as well as the fraction containing the polyclonal antibodies may be isolated from the serum. Immunoglobulin G or M can be prepared from a fraction which

5   recognizes only the polypeptide of the present invention using, for example, an affinity column coupled with the polypeptide of the present invention, and further purifying this fraction using protein A or protein G column.

To prepare monoclonal antibodies, immune cells are collected from the mammal immunized with the antigen and checked for the increased level of desired antibodies in the

10   serum as described above, and are subjected to cell fusion. The immune cells used for cell fusion are preferably obtained from spleen. Other preferred parental cells to be fused with the above immunocyte include, for example, myeloma cells of mammalians, and more preferably myeloma cells having an acquired property for the selection of fused cells by drugs.

15   The above immunocyte and myeloma cells can be fused according to known methods, for example, the method of Milstein et al. (Galfre and Milstein, Methods Enzymol 73: 3-46, 1981).

Resulting hybridomas obtained by the cell fusion may be selected by cultivating them in a standard selection medium, such as HAT medium (hypoxanthine, aminopterin,

20   and thymidine containing medium). The cell culture is typically continued in the HAT medium for several days to several weeks, the time being sufficient to allow all the other cells, with the exception of the desired hybridoma (non-fused cells), to die. Then, the standard limiting dilution is performed to screen and clone a hybridoma cell producing the desired antibody.

25   In addition to the above method, in which a non-human animal is immunized with an antigen for preparing hybridoma, human lymphocytes such as those infected by EB virus may be immunized with a polypeptide, polypeptide expressing cells, or their lysates *in vitro*. Then, the immunized lymphocytes are fused with human-derived myeloma cells that are capable of indefinitely dividing, such as U266, to yield a hybridoma producing a

30   desired human antibody that is able to bind to the polypeptide can be obtained (Unexamined Published Japanese Patent Application No. (JP-A) Sho 63-17688).

The obtained hybridomas are subsequently transplanted into the abdominal cavity of a mouse and the ascites are extracted. The obtained monoclonal antibodies can be purified by, for example, ammonium sulfate precipitation, a protein A or protein G column,

35   DEAE ion exchange chromatography, or an affinity column to which the polypeptide of the present invention is coupled. The antibody of the present invention can be used not

only for purification and detection of the polypeptide of the present invention, but also as a candidate for agonists and antagonists of the polypeptide of the present invention.   In addition, this antibody can be applied to the antibody treatment for diseases related to the polypeptide of the present invention.   When the obtained antibody is to be administered to

5   the human body (antibody treatment), a human antibody or a humanized antibody is preferable for reducing immunogenicity.

Forexample, transgenic animals having a repertory of human antibody genes may be immunized with an antigen selected from a polypeptide, polypeptide expressing cells, or their lysates.   Antibody producing cells are then collected from the animals and fused

10   with myeloma cells to obtain hybridoma, from which human antibodies against the polypeptide can be prepared (see WO92-03918, WO93-2227, WO94-02602, WO94-25585, WO96-33735, and WO96-34096).

Alternatively, an immune cell, such as an immunized lymphocyte, producing antibodies may be immortalized by an oncogene and used for preparing monoclonal

15   antibodies.

Monoclonal antibodies thus obtained can be also recombinantly prepared using genetic engineering techniques (see, for example, Borrebaeck and Larrick, Therapeutic Monoclonal Antibodies, published in the United Kingdom by MacMillan Publishers LTD, 1990).   For example, a DNA encoding an antibody may be cloned from an immune cell,

20   such as a hybridoma or an immunized lymphocyte producing the antibody, inserted into an appropriate vector, and introduced into host cells to prepare a recombinant antibody.   The present invention also provides recombinant antibodies prepared as described above.

Furthermore, an antibody of the present invention may be a fragment of an antibody or modified antibody, so long as it binds to one or more of the polypeptides of the

25   invention.   For instance, the antibody fragment may be Fab, F(ab')2, Fv, or single chain Fv (scFv), in which Fv fragments from H and L chains are ligated by an appropriate linker (Huston et al., Proc Natl Acad Sci USA 85: 5879-83, 1988).   More specifically, an antibody fragment may be generated by treating an antibody with an enzyme, such as papain or pepsin.   Alternatively, a gene encoding the antibody fragment may be

30   constructed, inserted into an expression vector, and expressed in an appropriate host cell (see, for example, Co et al., J Immunol 152: 2968-76, 1994; Better and Horwitz, Methods Enzymol 178: 476-96, 1989; Pluckthun and Skerra, Methods Enzymol 178: 497-515, 1989; Lamoyi, Methods Enzymol 121: 652-63, 1986; Rousseaux et al., Methods Enzymol 121: 663-9, 1986; Bird and Walker, Trends Biotechnol 9: 132-7, 1991).

35   An antibody may be modified by conjugation with a variety of molecules, such as polyethylene glycol (PEG).   The present invention provides for such modified antibodies.

The modified antibody can be obtained by chemically modifying an antibody.   These modification methods are conventional in the field.

Alternatively, an antibody of the present invention may be obtained as a chimeric antibody, between a variable region derived from nonhuman antibody and the constant region derived from human antibody, or as a humanized antibody, comprising the complementarity determining region (CDR) derived from nonhuman antibody, the frame work region (FR) derived from human antibody, and the constant region.   Such antibodies can be prepared by using known technology.

Antibodies obtained as above may be purified to homogeneity.   For example, the separation and purification of the antibody can be performed according to separation and purification methods used for general proteins.   For example, the antibody may be separated and isolated by the appropriately selected and combined use of column chromatographies, such as affinity chromatography, filter, ultrafiltration, salting-out, dialysis, SDS polyacrylamide gel electrophoresis, isoelectric focusing, and others (Antibodies: A Laboratory Manual. Ed Harlow and David Lane, Cold Spring Harbor Laboratory, 1988), but are not limited thereto.   A protein A column and protein G column can be used as the affinity column.   Exemplary protein A columns to be used include, for example, Hyper D, POROS, and Sepharose F.F. (Pharmacia).

Exemplary chromatography, with the exception of affinity includes, for example, ion-exchange chromatography, hydrophobic chromatography, gel filtration, reverse-phase chromatography, adsorption chromatography, and the like (Strategies for Protein Purification and Characterization: A Laboratory Course Manual. Ed Daniel R. Marshak et al., Cold Spring Harbor Laboratory Press, 1996).   The chromatographic procedures can be carried out by liquid-phase chromatography, such as HPLC, FPLC.

For example, measurement of absorbance, enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), radioimmunoassay (RIA), and/or immunofluorescence may be used to measure the antigen binding activity of the antibody of the invention.   In ELISA, the antibody of the present invention is immobilized on a plate, a polypeptide of the invention is applied to the plate, and then a sample containing a desired antibody, such as culture supernatant of antibody producing cells or purified antibodies, is applied.   Then, a secondary antibody that recognizes the primary antibody and is labeled with an enzyme, such as alkaline phosphatase, is applied, and the plate is incubated.   Next, after washing, an enzyme substrate, such as p-nitrophenyl phosphate, is added to the plate, and the absorbance is measured to evaluate the antigen binding activity of the sample.   A fragment of the polypeptide, such as a C-terminal or N-terminal fragment, may be used as the antigen to evaluate the binding activity of the antibody.

BIAcore (Pharmacia) may be used to evaluate the activity of the antibody according to the present invention.

The above methods allow for the detection or measurement of the polypeptide of the invention, by exposing the antibody of the invention to a sample assumed to contain the polypeptide of the invention, and detecting or measuring the immune complex formed by the antibody and the polypeptide.

Because the method of detection or measurement of the polypeptide according to the invention can specifically detect or measure a polypeptide, the method may be useful in a variety of experiments in which the polypeptide is used.

The present invention also provides a polynucleotide which hybridizes with the polynucleotide encoding human *RHBDF1* protein (SEQ ID NO: 15) or the complementary strand thereof, and which comprises at least 15 nucleotides. The polynucleotide of the present invention is preferably a polynucleotide which specifically hybridizes with the DNA encoding the polypeptide of the present invention. The term "specifically hybridize" as used herein, means that cross-hybridization does not occur significantly with DNA encoding other proteins, under the usual hybridizing conditions, preferably under stringent hybridizing conditions. Such polynucleotides include, probes, primers, nucleotides and nucleotide derivatives (for example, antisense oligonucleotides and ribozymes), which specifically hybridize with DNA encoding the polypeptide of the invention or its complementary strand. Moreover, such polynucleotide can be utilized for the preparation of DNA array.

The present invention includes an antisense oligonucleotide that hybridizes with any site within the nucleotide sequence of SEQ ID NO: 15. This antisense oligonucleotide is preferably against at least 15 continuous nucleotides of the nucleotide sequence of SEQ ID NO: 15. The above-mentioned antisense oligonucleotide, which contains an initiation codon in the above-mentioned at least 15 continuous nucleotides, is even more preferred. More specifically, such antisense oligonucleotides include those comprising the nucleotide sequence of SEQ ID NO: 11 for suppressing the expression of *RHBDF1*.

Derivatives or modified products of antisense oligonucleotides can be used as antisense oligonucleotides. Examples of such modified products include lower alkyl phosphonate modifications such as methyl-phosphonate-type or ethyl-phosphonate-type, phosphorothioate modifications and phosphoroamidate modifications.

The term "antisense oligonucleotides" as used herein means, not only those in which the nucleotides corresponding to those constituting a specified region of a DNA or mRNA are entirely complementary, but also those having a mismatch of one or more

nucleotides, as long as the DNA or mRNA and the antisense oligonucleotide can specifically hybridize with the nucleotide sequence of SEQ ID NO: 15.

Such polynucleotides are contained as those having, in the "at least 15 continuous nucleotide sequence region", a homology of at least 70% or higher, preferably at 80% or

5   higher, more preferably 90% or higher, even more preferably 95% or higher.   The algorithm stated herein can be used to determine the homology.   Such polynucleotides are useful as probes for the isolation or detection of DNA encoding the polypeptide of the invention as stated in a later example or as a primer used for amplifications.

The antisense oligonucleotide derivatives of the present invention act upon cells

10   producing the polypeptide of the invention by binding to the DNA or mRNA encoding the polypeptide, inhibiting its transcription or translation, promoting the degradation of the mRNA, and inhibiting the expression of the polypeptide of the invention, thereby resulting in the inhibition of the polypeptide's function.

An antisense oligonucleotide derivative of the present invention can be made into

15   an external preparation, such as a liniment or a poultice, by mixing with a suitable base material which is inactive against the derivatives.

Also, as needed, the derivatives can be formulated into tablets, powders, granules, capsules, liposome capsules, injections, solutions, nose-drops and freeze-drying agents by adding excipients, isotonic agents, solubilizers, stabilizers, preservatives, pain-killers, and

20   such.   These can be prepared by following usual methods.

The antisense oligonucleotide derivative is given to the patient by directly applying onto the ailing site or by injecting into a blood vessel so that it will reach the site of ailment. An antisense-mounting medium can also be used to increase durability and membrane-permeability.   Examples are, liposome, poly-L-lysine, lipid, cholesterol,

25   lipofectin or derivatives of these.

The dosage of the antisense oligonucleotide derivative of the present invention can be adjusted suitably according to the patient's condition and used in desired amounts.   For example, a dose range of 0.1 to 100 mg/kg, preferably 0.1 to 50 mg/kg can be administered.

30   The present invention also includes small interfering RNAs (siRNA) comprising a combination of a sense strand nucleic acid and an antisense strand nucleic acid of the nucleotide sequence of SEQ ID NO: 15.   More specifically, such siRNA for suppressing the expression of *RHBDF1* include those whose sense strand comprises the nucleotide sequence of SEQ ID NO: 13.

35   The term "siRNA" refers to a double stranded RNA molecule which prevents translation of a target mRNA.   Standard techniques are used for introducing siRNA into

cells, including those wherein DNA is used as the template to transcribe RNA.   The
siRNA comprises a sense nucleic acid sequence and an anti-sense nucleic acid sequence of
the polynucleotide encoding human *RHBDF1* protein (SEQ ID NO: 15).   The siRNA is
constructed such that a single transcript (double stranded RNA) has both the sense and
5   complementary antisense sequences from the target gene, *e.g.*, a hairpin.

The method is used to alter gene expression of a cell, i.e., up-regulate the
expression of *RHBDF1*, *e.g.*, as a result of malignant transformation of the cells.   Binding
of the siRNA to *RHBDF1* transcript in the target cell results in a reduction of protein
production by the cell.   The length of the oligonucleotide is at least 10 nucleotides and
10   may be as long as the naturally occurring the transcript.   Preferably, the oligonucleotide
is19-25 nucleotides in length.   Most preferably, the oligonucleotide is less than 75, 50, 25
nucleotides in length.

The nucleotide sequence of siRNAs may be designed using a siRNA design
computer program available from the Ambion website (http://www.ambion.com/techli
15   b/misc/siRNA_finder.html).   Nucleotide sequences for the siRNA are selected by t
he computer program based on the following protocol:

Selection of siRNA Target Sites:

1.   Beginning with the AUG start codon of the object transcript, scan downstream for
     AA dinucleotide sequences.   Record the occurrence of each AA and the 3' adjacent
20   19 nucleotides as potential siRNA target sites.   Tuschl, et al. recommend against
     designing siRNA to the 5' and 3' untranslated regions (UTRs) and regions near the
     start codon (within 75 bases) as these may be richer in regulatory protein binding sites.
     UTR-binding proteins and/or translation initiation complexes may interfere with the
     binding of the siRNA endonuclease complex.

25   2.   Compare the potential target sites to the human genome database and eliminate
     from consideration any target sequences with significant homology to other coding
     sequences.   The homology search can be performed using BLAST, which can be
     found on the NCBI server at: www.ncbi.nlm.nih.gov/BLAST/

3.   Select qualifying target sequences for synthesis.   At Ambion, preferably several
30   target sequences can be selected along the length of the gene for evaluation.

The antisense oligonucleotide or siRNA of the invention inhibit the expression of
the polypeptide of the invention and is thereby useful for suppressing the biological
activity of the polypeptide of the invention.   Also, expression-inhibitors, comprising the
antisense oligonucleotide or siRNA of the invention, are useful in the point that they can
35   inhibit the biological activity of the polypeptide of the invention.   Therefore, a
composition comprising the antisense oligonucleotide or siRNA of the present invention is

useful in treating a cell proliferative disease such as cancer.

Moreover, the present invention provides a method for diagnosing a cell proliferative disease using the expression level of the polypeptides of the present invention as a diagnostic marker.

5   This diagnosing method comprises the steps of: (a) detecting the expression level of the *RHBDF1* gene of the present invention; and (b) relating an elevation of the expression level to the cell proliferative disease, such as cancer.

The expression levels of the the*RHBDF1* gene in a particular specimen can be estimated by quantifying mRNA corresponding to or protein encoded by the *RHBDF1*

10   gene.   Quantification methods for mRNA are known to those skilled in the art.   For example, the levels of mRNAs corresponding to the *RHBDF1* gene can be estimated by Northern blotting or RT-PCR.   Since the full-length nucleotide sequences of the *RHBDF1* genes are shown in SEQ ID NO: 15, anyone skilled in the art can design the nucleotide sequences for probes or primers to quantify the *RHBDF1* gene.

15   Also the expression level of the *RHBDF1* gene can be analyzed based on activity or quantity of protein encoded by the gene.   A method for determining the quantity of the *RHBDF1* protein is shown in bellow.   For example, immunoassay method is useful for determination of the protein in biological material.   Any biological materials can be used for the determination of the protein or it's activity.   For example, blood sample is analyzed

20   for estimation of the protein encoded by serum marker.   Another hand, a suitable method can be selected for the determination of the activity protein encoded by the *RHBDF1* gene according to the activity of the protein to be analyzed.

Expression levels of the *RHBDF1* gene in a specimen (test sample) are estimated and compared with those in a normal sample.   When such a comparison shows that the

25   expression level of the target gene is higher than those in the normal sample, the subject is judged to be affected with a cell proliferative disease.   The expression level of *RHBDF1* gene in the specimens from the normal sample and subject may be determined at the same time.   Alternatively, normal ranges of the expression levels can be determined by a statistical method based on the results obtained by analyzing the expression level of the

30   gene in specimens previously collected from a control group.   A result obtained by examining the sample of a subject is compared with the normal range; when the result does not fall within the normal range, the subject is judged to be affected with the cell proliferative disease.   In the present invention, the cell proliferative disease to be diagnosed is preferably cancer.   More preferably, when the expression level of the

35   *RHBDF1* gene is estimated and compared with those in a normal sample, the cell proliferative disease to be diagnosed is any one of CML, AML, or lung adenocarcinoma.

In the present invention, a diagnostic agent for diagnosing cell proliferative disease, such as cancer including CML, AML, or lung adenocarcinoma, is also provided. The diagnostic agent of the present invention comprises a compound that binds to the polynucleotide or the polypeptide of the present invention. Preferably, the

5   oligonucleotide that hybridizes to the polynucleotide of the present invention, or the antibodies that bind to the polypeptide of the present invention may be used as these compound.

       Moreover, the present invention provides a method of screening for a compound for treating a cell proliferative disease using the polypeptide of the present invention. An

10   embodiment of this screening method comprises the steps of: (a) contacting a test compound with a polypeptide of the present invention, (b) detecting the binding activity between the polypeptide of the present invention and the test compound, and (c) selecting a compound that binds to the polypeptide of the present invention.

       The polypeptide of the present invention to be used for screening may be a

15   recombinant polypeptide or a protein derived from the nature, or a partial peptide thereof. Any test compound, for example, cell extracts, cell culture supernatant, products of fermenting microorganism, extracts from marine organism, plant extracts, purified or crude proteins, peptides, non-peptide compounds, synthetic micromolecular compounds and natural compounds, can be used. The polypeptide of the present invention to be contacted

20   with a test compound can be, for example, a purified polypeptide, a soluble protein, a form bound to a carrier, or a fusion protein fused with other polypeptides.

       As a method of screening for proteins, for example, that bind to the polypeptide of the present invention using the polypeptide of the present invention, many methods well known by a person skilled in the art can be used. Such a screening can be conducted by,

25   for example, immunoprecipitation method, specifically, in the following manner. The gene encoding the polypeptide of the present invention is expressed in animal cells and so on by inserting the gene to an expression vector for foreign genes, such as pSV2neo, pcDNA I, and pCD8. The promoter to be used for the expression may be any promoter that can be used commonly and include, for example, the SV40 early promoter (Rigby in

30   Williamson (ed.), Genetic Engineering, vol. 3. Academic Press, London, 83-141, 1982), the EF-1α promoter (Kim et al., Gene 91: 217-23, 1990), the CAG promoter (Niwa et al., Gene 108: 193-200, 1991), the RSV LTR promoter (Cullen, Methods in Enzymology 152: 684-704, 1987) the SRα promoter (Takebe et al., Mol Cell Biol 8: 466, 1988), the CMV immediate early promoter (Seed and Aruffo, Proc Natl Acad Sci USA 84: 3365-9, 1987),

35   the SV40 late promoter (Gheysen and Fiers, J Mol Appl Genet 1: 385-94, 1982), the Adenovirus late promoter (Kaufman et al., Mol Cell Biol 9: 946 (1989)), the HSV TK

promoter, and so on.   The introduction of the gene into animal cells to express a foreign gene can be performed according to any methods, for example, the electroporation method (Chu et al., Nucleic Acids Res 15: 1311-26, 1987), the calcium phosphate method (Chen and Okayama, Mol Cell Biol 7: 2745-52, 1987), the DEAE dextran method (Lopata et al.,

5   Nucleic Acids Res 12: 5707-17, 1984; Sussman and Milman, Mol Cell Biol 4: 1642-3, 1985), the Lipofectin method (Derijard, B Cell 7: 1025-37, 1994; Lamb et al., Nature Genetics 5: 22-30, 1993: Rabindran et al., Science 259: 230-4, 1993), and so on.   The polypeptide of the present invention can be expressed as a fusion protein comprising a recognition site (epitope) of a monoclonal antibody by introducing the epitope of the

10   monoclonal antibody, whose specificity has been revealed, to the N- or C- terminus of the polypeptide of the present invention.   A commercially available epitope-antibody system can be used (Experimental Medicine 13: 85-90, 1995).   Vectors which can express a fusion protein with, for example, β-galactosidase, maltose binding protein, glutathione S-transferase, green florescence protein (GFP) and so on by the use of its multiple cloning

15   sites are commercially available.

A fusion protein prepared by introducing only small epitopes consisting of several to a dozen amino acids so as not to change the property of the polypeptide of the present invention by the fusion is also reported.   Epitopes, such as polyhistidine (His-tag), influenza aggregate HA, human c-myc, FLAG, Vesicular stomatitis virus glycoprotein

20   (VSV-GP), T7 gene 10 protein (T7-tag), human simple herpes virus glycoprotein (HSV-tag), E-tag (an epitope on monoclonal phage), and such, and monoclonal antibodies recognizing them can be used as the epitope-antibody system for screening proteins binding to the polypeptide of the present invention (Experimental Medicine 13: 85-90, 1995).

25   In immunoprecipitation, an immune complex is formed by adding these antibodies to cell lysate prepared using an appropriate detergent.   The immune complex consists of the polypeptide of the present invention, a polypeptide comprising the binding ability with the polypeptide, and an antibody.   Immunoprecipitation can be also conducted using antibodies against the polypeptide of the present invention, besides using antibodies

30   against the above epitopes, which antibodies can be prepared as described above.

An immune complex can be precipitated, for example by Protein A sepharose or Protein G sepharose when the antibody is a mouse IgG antibody.   If the polypeptide of the present invention is prepared as a fusion protein with an epitope, such as GST, an immune complex can be formed in the same manner as in the use of the antibody against the

35   polypeptide of the present invention, using a substance specifically binding to these epitopes, such as glutathione-Sepharose 4B.

Immunoprecipitation can be performed by following or according to, for example, the methods in the literature (Harlow and Lane, Antibodies, 511-52, Cold Spring Harbor Laboratory publications, New York (1988)).

SDS-PAGE is commonly used for analysis of immunoprecipitated proteins and the
5 bound protein can be analyzed by the molecular weight of the protein using gels with an appropriate concentration. Since the protein bound to the polypeptide of the present invention is difficult to detect by a common staining method, such as Coomassie staining or silver staining, the detection sensitivity for the protein can be improved by culturing cells in culture medium containing radioactive isotope, ^{35}S-methionine or ^{35}S-cystein,
10 labeling proteins in the cells, and detecting the proteins. The target protein can be purified directly from the SDS-polyacrylamide gel and its sequence can be determined, when the molecular weight of a protein has been revealed.

As a method for screening proteins binding to the polypeptide of the present invention using the polypeptide, for example, West-Western blotting analysis (Skolnik et
15 al., Cell 65: 83-90 (1991)) can be used. Specifically, a protein binding to the polypeptide of the present invention can be obtained by preparing a cDNA library from cells, tissues, organs (for example, trachea, thyroid, spinal cord, prostate, skeletal muscle, or placenta), or cultured cells expected to express a protein binding to the polypeptide of the present invention using a phage vector (e.g., ZAP), expressing the protein on LB-agarose, fixing
20 the protein expressed on a filter, reacting the purified and labeled polypeptide of the present invention with the above filter, and detecting the plaques expressing proteins bound to the polypeptide of the present invention according to the label. The polypeptide of the invention may be labeled by utilizing the binding between biotin and avidin, or by utilizing an antibody that specifically binds to the polypeptide of the present invention, or a peptide
25 or polypeptide (for example, GST) that is fused to the polypeptide of the present invention. Methods using radioisotope or fluorescence and such may be also used.

Alternatively, in another embodiment of the screening method of the present invention, a two-hybrid system utilizing cells may be used ("MATCHMAKER Two-Hybrid system", "Mammalian MATCHMAKER Two-Hybrid Assay Kit",
30 "MATCHMAKER one-Hybrid system" (Clontech); "HybriZAP Two-Hybrid Vector System" (Stratagene); the references "Dalton and Treisman, Cell 68: 597-612 (1992)", "Fields and Sternglanz, Trends Genet 10: 286-92 (1994)").

In the two-hybrid system, the polypeptide of the invention is fused to the SRF-binding region or GAL4-binding region and expressed in yeast cells. A cDNA
35 library is prepared from cells expected to express a protein binding to the polypeptide of the invention, such that the library, when expressed, is fused to the VP16 or GAL4

transcriptional activation region.   The cDNA library is then introduced into the above
yeast cells and the cDNA derived from the library is isolated from the positive clones
detected (when a protein binding to the polypeptide of the invention is expressed in yeast
cells, the binding of the two activates a reporter gene, making positive clones detectable).

5   A protein encoded by the cDNA can be prepared by introducing the cDNA isolated above
to *E. coli* and expressing the protein.

       As a reporter gene, for example, Ade2 gene, lacZ gene, CAT gene, luciferase gene
and such can be used besides HIS3 gene.

       A compound binding to the polypeptide of the present invention can also be

10   screened using affinity chromatography.   For example, the polypeptide of the invention
may be immobilized on a carrier of an affinity column, and a test compound, containing a
protein capable of binding to the polypeptide of the invention, is applied to the column.   A
test compound herein may be, for example, cell extracts, cell lysates, etc.   After loading
the test compound, the column is washed, and compounds bound to the polypeptide of the

15   invention can be prepared.

       When the test compound is a protein, the amino acid sequence of the obtained
protein is analyzed, an oligo DNA is synthesized based on the sequence, and cDNA
libraries are screened using the oligo DNA as a probe to obtain a DNA encoding the
protein.

20   A biosensor using the surface plasmon resonance phenomenon may be used as a
mean for detecting or quantifying the bound compound in the present invention.   When
such a biosensor is used, the interaction between the polypeptide of the invention and a test
compound can be observed real-time as a surface plasmon resonance signal, using only a
minute amount of polypeptide and without labeling (for example, BIAcore, Pharmacia).

25   Therefore, it is possible to evaluate the binding between the polypeptide of the invention
and a test compound using a biosensor such as BIAcore.

       The methods of screening for molecules that bind when the immobilized
polypeptide of the present invention is exposed to synthetic chemical compounds, or
natural substance banks, or a random phage peptide display library, or the methods of

30   screening using high-throughput based on combinatorial chemistry techniques (Wrighton
et al., Science 273: 458-64 (1996); Verdine, Nature 384: 11-13 (1996); Hogan, Nature 384:
17-9 (1996)) to isolate not only proteins but chemical compounds that bind to protein of
the present invention (including agonist and antagonist) are well known to one skilled in
the art.

35   Alternatively, the screening method of the present invention may comprise the
following steps:

a) contacting a candidate compound with a cell into which a vector comprising the transcriptional regulatory region of a marker gene and a reporter gene that is expressed under the control of the transcriptional regulatory region has been introduced, wherein the marker genes comprising nucleotide sequence of SEQ ID:NO 15

5

b) measuring the activity of said reporter gene; and

c) selecting a compound that reduces the expression level of said reporter gene in comparison with the expression level of said reporter gene detected in the absence of the test compound.

10   Suitable reporter genes and host cells are well known in the art. The reporter construct required for the screening can be prepared by using the transcriptional regulatory region of a marker gene. When the transcriptional regulatory region of a marker gene has been known to those skilled in the art, a reporter construct can be prepared by using the previous sequence information. When the transcriptional regulatory region of a marker

15 gene remains unidentified, a nucleotide segment containing the transcriptional regulatory region can be isolated from a genome library based on the nucleotide sequence information of the marker gene.

    A compound isolated by the screening is a candidate for drugs which promote or inhibit the activity of the polypeptide of the present invention, for treating or preventing

20 diseases attributed to, for example, cell proliferative diseases, such as cancer. A compound in which a part of the structure of the compound obtained by the present screening method having the activity of binding to the polypeptide of the present invention is converted by addition, deletion and/or replacement, is included in the compounds obtained by the screening method of the present invention.

25   In a further embodiment, the present invention provides methods for screening candidate agents which are potential targets in the treatment of cell proliferative disease. As discussed in detail above, by controlling the expression levels of the *RHBDF1*, one can control the onset and progression of either CML, AML, or lung adenocatcinoma. Thus, candidate agents, which are potential targets in the treatment of cell proliferative disease,

30 can be identified through screenings that use the expression levels and activities of *RHBDF1* as indices. In the context of the present invention, such screening may comprise, for example, the following steps:

a) contacting a candidate compound with a cell expressing the *RHBDF1*; and

b) selecting a compound that reduces the expression level of *RHBDF1* in comparison

35   with the expression level detected in the absence of the test compound.

    Cells expressing at least one of the *RHBDF1* include, for example, cell lines

established from CML, AML, and lung adenocarcinoma ; such cells can be used for the
above screening of the present invention.   The expression level can be estimated by
methods well known to one skilled in the art.   In the method of screening, a compound
that reduces the expression level of at least one of *RHBDF1* can be selected as candidate
5   agents.

In another embodiment of the method for screening a compound for treating a cell
proliferative disease of the present invention, the method utilizes biological activity of the
polypeptide of the present invention as an index.   Since the *RHBDF1* proteins of the
present invention have the activity of promoting cell proliferation, a compound which
10   promotes or inhibits this activity of one of these proteins of the present invention can be
screened using this activity as an index.   This screening method includes the steps of: (a)
contacting a test compound with the polypeptide of the present invention; (b) detecting the
biological activity of the polypeptide of step (a); and (c) selecting a compound that
suppresses the biological activity of the polypeptide in comparison with the biological
15   activity detected in the absence of the test compound.

Any polypeptides can be used for screening so long as they comprise the biological
activity of the *RHBDF1* protein.   Such biological activity includes cell-proliferating
activity of the human *RHBDF1* protein.

Any test compounds, for example, cell extracts, cell culture supernatant, products
20   of fermenting microorganism, extracts of marine organism, plant extracts, purified or crude
proteins, peptides, non-peptide compounds, synthetic micromolecular compounds, natural
compounds, can be used.

The compound isolated by this screening is a candidate for agonists or antagonists
of the polypeptide of the present invention.   The term "agonist" refers to molecules that
25   activate the function of the polypeptide of the present invention by binding thereto.
Likewise, the term "antagonist" refers to molecules that inhibit the function of the
polypeptide of the present invention by binding thereto.   Moreover, a compound isolated
by this screening is a candidate for compounds which inhibit the *in vivo* interaction of the
polypeptide of the present invention with molecules (including DNAs and proteins).

30   When the biological activity to be detected in the present method is cell
proliferation, it can be detected, for example, by preparing cells which express the
polypeptide of the present invention, culturing the cells in the presence of a test compound,
and determining the speed of cell proliferation, measuring the cell cycle and such, as well
as by measuring the colony forming activity as described in the Examples.

35   The compound isolated by the above screenings is a candidate for drugs which
inhibit the activity of the polypeptide of the present invention and can be applied to the

treatment of diseases associated with the polypeptide of the present invention, for example, cell proliferative diseases including cancer. More particularly, when the biological activity of *RHBDF1* protein is used as the index, compounds screened by the present method serve as a candidate for drugs for the treatment of CML, AML, lung

5   adenocarcinoma.

Moreover, compound in which a part of the structure of the compound inhibiting the activity of *RHBDF1* protein is converted by addition, deletion and/or replacement are also included in the compounds obtainable by the screening method of the present invention.

10   When administrating the compound isolated by the methods of the invention as a pharmaceutical for humans and other mammals, such as mice, rats, guinea-pigs, rabbits, chicken, cats, dogs, sheep, pigs, cattle, monkeys, baboons, chimpanzees, for treating a cell proliferative disease (e.g., cancer) the isolated compound can be directly administered or can be formulated into a dosage form using known pharmaceutical preparation methods.

15   For example, according to the need, the drugs can be taken orally, as sugarcoated tablets, capsules, elixirs and microcapsules, or non-orally, in the form of injections of sterile solutions or suspensions with water or any other pharmaceutically acceptable liquid. For example, the compounds can be mixed with pharmacologically acceptable carriers or medium, specifically, sterilized water, physiological saline, plant-oil, emulsifiers,

20   suspending agents, surfactants, stabilizers, flavoring agents, excipients, vehicles, preservatives, binders and such, in a unit dose form required for generally accepted drug implementation. The amount of active ingredients in these preparations makes a suitable dosage within the indicated range acquirable.

Examples of additives that can be mixed to tablets and capsules are, binders such as

25   gelatin, corn starch, tragacanth gum and arabic gum; excipients such as crystalline cellulose; swelling agents such as corn starch, gelatin and alginic acid; lubricants such as magnesium stearate; sweeteners such as sucrose, lactose or saccharin; flavoring agents such as peppermint, Gaultheria adenothrix oil and cherry. When the unit dosage form is a capsule, a liquid carrier, such as oil, can also be further included in the above ingredients.

30   Sterile composites for injections can be formulated following normal drug implementations using vehicles such as distilled water used for injections.

Physiological saline, glucose, and other isotonic liquids including adjuvants, such as D-sorbitol, D-mannnose, D-mannitol, and sodium chloride, can be used as aqueous solutions for injections. These can be used in conjunction with suitable solubilizers, such

35   as alcohol, specifically ethanol, polyalcohols such as propylene glycol and polyethylene glycol, non-ionic surfactants, such as Polysorbate 80 (TM) and HCO-50.

Sesame oil or Soy-bean oil can be used as a oleaginous liquid and may be used in conjunction with benzyl benzoate or benzyl alcohol as a solubilizers and may be formulated with a buffer, such as phosphate buffer and sodium acetate buffer; a pain-killer, such as procaine hydrochloride; a stabilizer, such as benzyl alcohol, phenol; and an

5   anti-oxidant.   The prepared injection may be filled into a suitable ampule.

Methods well known to one skilled in the art may be used to administer the inventive pharmaceutical compound to patients, for example as intraarterial, intravenous, percutaneous injections and also as intranasal, transbronchial, intramuscular or oral administrations.   The dosage and method of administration vary according to the

10   body-weight and age of a patient and the administration method; however, one skilled in the art can routinely select them.   If said compound is encodable by a DNA, the DNA can be inserted into a vector for gene therapy and the vector administered to perform the therapy.   The dosage and method of administration vary according to the body-weight, age, and symptoms of a patient but one skilled in the art can select them suitably.

15   For example, although there are some differences according to the symptoms, the dose of a compound that binds with the polypeptide of the present invention and regulates its activity is about 0.1 mg to about 100 mg per day, preferably about 1.0 mg to about 50 mg per day and more preferably about 1.0 mg to about 20 mg per day, when administered orally to a normal adult (weight 60 kg).

20   When administering parenterally, in the form of an injection to a normal adult (weight 60 kg), although there are some differences according to the patient, target organ, symptoms and method of administration, it is convenient to intravenously inject a dose of about 0.01 mg to about 30 mg per day, preferably about 0.1 to about 20 mg per day and more preferably about 0.1 to about 10 mg per day.   Also, in the case of other animals too,

25   it is possible to administer an amount converted to 60kgs of body-weight.

Moreover, the present invention provides a method for treating or preventing a cell proliferative disease, such as cancer, using an antibody against the polypeptide of the present invention.   According to the method, a pharmaceutically effective amount of an antibody against the polypeptide of the present invention is administered.   Since the

30   expression of the *RHBDF1* protein are up-regulated in cancer cells, and the suppression of the expression of these proteins leads to the decrease in cell proliferating activity, it is expected that cell proliferative diseases can be treated or prevented by binding the antibody and these proteins.   Thus, an antibody against the polypeptide of the present invention are administered at a dosage sufficient to reduce the activity of the protein of the present

35   invention, which is in the range of   3 mg to 2000 mg per day (60 kg of body-weight).
For example, although there are some differences according to the symptoms, the dose of

the antibodies that binds with the polypeptide of the present invention is about 5 mg to about 1000 mg per day, and preferably about 10 mg to about 500 mg per day to a normal adult (weight 60 kg).

5   Alternatively, an antibody binding to a cell surface marker specific for tumor cells can be used as a tool for drug delivery. For example, an antibody conjugated with a cytotoxic agent is administered at a dosage sufficient to injure tumor cells.

The present invention also relates to a method of inducing anti-tumor immunity comprising the step of administering *RHBDF1* protein or an immunologically active fragment thereof, or a polynucleotide encoding the protein or fragments thereof. The

10   *RHBDF1* protein or the immunologically active fragments thereof are useful as vaccines against cell proliferative diseases. In some cases the proteins or fragments thereof may be administered in a form bound to the T cell recepor (TCR) or presented by an antigen presenting cell (APC), such as macrophage, dendritic cell (DC), or B-cells. Due to the strong antigen presenting ability of DC, the use of DC is most preferable among the APCs.

15   In the present invention, vaccine against cell proliferative diseases refers to a substance that has the function to induce anti-tumor immunity upon inoculation into animals. In general, anti-tumor immunity includes immune responses such as follows:
-   induction of cytotoxic lymphocytes against tumors,
-   induction of antibodies that recognize tumors, and

20   -   induction of anti-tumor cytokine production.

Therefore, when a certain protein induces any one of these immune responses upon inoculation into an animal, the protein is decided to have anti-tumor immunity inducing effect. The induction of the anti-tumor immunity by a protein can be detected by observing *in vivo* or *in vitro* the response of the immune system in the host against the

25   protein.

For example, a method for detecting the induction of cytotoxic T lymphocytes is well known. A foreign substance that enters the living body is presented to T cells and B cells by the action of antigen presenting cells (APCs). T cells that respond to the antigen presented by APC in antigen specific manner differentiate into cytotoxic T cells (or

30   cytotoxic T lymphocytes; CTLs) due to stimulation by the antigen, and then proliferate (this is referred to as activation of T cells). Therefore, CTL induction by a certain peptide can be evaluated by presenting the peptide to T cell by APC, and detecting the induction of CTL. Furthermore, APC has the effect of activating CD4+ T cells, CD8+ T cells, macrophages, eosinophils, and NK cells. Since CD4+ T cells and CD8+ T cells are also

35   important in anti-tumor immunity, the anti-tumor immunity inducing action of the peptide can be evaluated using the activation effect of these cells as indicators.

A method for evaluating the inducing action of CTL using dendritic cells (DCs) as APC is well known in the art. DC is a representative APC having the strongest CTL inducing action among APCs. In this method, the test polypeptide is initially contacted with DC, and then this DC is contacted with T cells. Detection of T cells having

5   cytotoxic effects against the cells of interest after the contact with DC shows that the test polypeptide has an activity of inducing the cytotoxic T cells. Activity of CTL against tumors can be detected, for example, using the lysis of ^{51}Cr-labeled tumor cells as the indicator. Alternatively, the method of evaluating the degree of tumor cell damage using ^{3}H-thymidine uptake activity or LDH (lactose dehydrogenase)-release as the indicator is

10   also well known.

Apart from DC, peripheral blood mononuclear cells (PBMCs) may also be used as the APC. The induction of CTL is reported that the it can be enhanced by culturing PBMC in the presence of GM-CSF and IL-4. Similarly, CTL has been shown to be induced by culturing PBMC in the presence of keyhole limpet hemocyanin (KLH) and

15   IL-7.

The test polypeptides confirmed to possess CTL inducing activity by these methods are polypeptides having DC activation effect and subsequent CTL inducing activity. Therefore, polypeptides that induce CTL against tumor cells are useful as vaccines against tumors. Furthermore, APC that acquired the ability to induce CTL

20   against tumors by contacting with the polypeptides are useful as vaccines against tumors. Furthermore, CTL that acquired cytotoxicity due to presentation of the polypeptide antigens by APC can be also used as vaccines against tumors. Such therapeutic methods for tumors using anti-tumor immunity due to APC and CTL are referred to as cellular immunotherapy.

25   Generally, when using a polypeptide for cellular immunotherapy, efficiency of the CTL-induction is known to increase by combining a plurality of polypeptides having different structures and contacting them with DC. Therefore, when stimulating DC with protein fragments, it is advantageous to use a mixture of multiple types of fragments.

Alternatively, the induction of anti-tumor immunity by a polypeptide can be

30   confirmed by observing the induction of antibody production against tumors. For example, when antibodies against a polypeptide are induced in a laboratory animal immunized with the polypeptide, and when growth of tumor cells is suppressed by those antibodies, the polypeptide can be determined to have an ability to induce anti-tumor immunity.

35   Anti-tumor immunity is induced by administering the vaccine of this invention, and the induction of anti-tumor immunity enables treatment and prevention of cell

proliferating diseases, such as CML, AML, or lung adenocarcinoma.   Therapy against cancer or prevention of the onset of cancer includes any of the steps, such as inhibition of the growth of cancerous cells, involution of cancer, and suppression of occurrence of cancer.   Decrease in mortality of individuals having cancer, decrease of tumor markers in

5   the blood, alleviation of detectable symptoms accompanying cancer, and such are also included in the therapy or prevention of cancer.   Such therapeutic and preventive effects are preferably statistically significant.   For example, in observation, at a significance level of 5% or less, wherein the therapeutic or preventive effect of a vaccine against cell proliferative diseases is compared to a control without vaccine administration.   For

10   example, Student's t-test, the Mann-Whitney U-test, or ANOVA may be used for statistical analyses.

The above-mentioned protein having immunological activity or a vector encoding the protein may be combined with an adjuvant.   An adjuvant refers to a compound that enhances the immune response against the protein when administered together (or

15   successively) with the protein having immunological activity.   Examples of adjuvants include cholera toxin, salmonella toxin, alum, and such, but are not limited thereto. Furthermore, the vaccine of this invention may be combined appropriately with a pharmaceutically acceptable carrier.   Examples of such carriers are sterilized water, physiological saline, phosphate buffer, culture fluid, and such.   Furthermore, the vaccine

20   may contain as necessary, stabilizers, suspensions, preservatives, surfactants, and such. The vaccine is administered systemically or locally.   Vaccine administration may be performed by single administration, or boosted by multiple administrations.

When using APC or CTL as the vaccine of this invention, tumors can be treated or prevented, for example, by the *ex vivo* method.   More specifically, PBMCs of the subject

25   receiving treatment or prevention are collected, the cells are contacted with the polypeptide *ex vivo*, and following the induction of APC or CTL, the cells may be administered to the subject.   APC can be also induced by introducing a vector encoding the polypeptide into PBMCs *ex vivo*.   APC or CTL induced in vitro can be cloned prior to administration.   By cloning and growing cells having high activity of damaging target cells, cellular

30   immunotherapy can be performed more effectively.   Furthermore, APC and CTL isolated in this manner may be used for cellular immunotherapy not only against individuals from whom the cells are derived, but also against similar types of tumors from other individuals.

Furthermore, a pharmaceutical composition for treating or preventing a cell proliferative disease, such as cancer, comprising a pharmaceutically effective amount of

35   the polypeptide of the present invention is provided.   The pharmaceutical composition may be used for raising anti tumor immunity.   The normal expression of *RHBDF1*,

restricted to trachea, thyroid, spinal cord, prostate, skeletal muscle, or placenta.   Therefore, suppression of these genes may not adversely affect other organs.   Thus, the *RHBDF1* polypeptides are preferable for treating cell proliferative disease, especially CML, AML, or lung adenocarcinoma.

5   The following examples are presented to illustrate the present invention and to assist one of ordinary skill in making and using the same.   The examples are not intended in any way to otherwise limit the scope of the invention.

        Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this

10   invention belongs.   Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below.   Any patents, patent applications, and publications cited herein are incorporated by reference.

15   Best Mode for Carrying out the Invention

        The present invention is illustrated in details by following Examples, but is not restricted to these Examples.

Materials and Methods

Cell lines and clinical materials

20   Human leukemia K562 cells were kindly provided from Dr. M. Towatari (Nagoya Univ., School of Med., Nagoya, Japan).   Human lung cancer lines A549, LC319 and H522, and a mouse fibroblast cell line NIH3T3 were purchased from the American Type Culture Collection (ATCC, Rockville, MD).   All cells were cultured in appropriate media; i.e. RPMI-1640 (Sigma, St. Louis, MO) for K562, A549, LC319 and H522; Dulbecco's

25   modified Eagle's medium (Invitrogen, Carlsbad, CA) for NIH3T3, each supplemented with 10% fetal bovine serum (Cansera) and 1% antibiotic/antimycotic solution (Sigma).   Cells were maintained at 37^0C in an atmosphere of humidified air with 5% CO_2.   Acute myeloid leukemia and lung cancer samples were obtained from patients with written informed consent.

30

Isolation of a novel human gene, C6135 by using cDNA microaray

        Fabrication of the cDNA microarray slides has been described (Ono *et al.*, 2000. *Cancer Res.*, 60, 5007-11.).   For each analysis of expression profiles, duplicate sets of cDNA microarray slides containing 23,040 cDNA spots was   prepared, to reduce

35   experimental fluctuation.   Briefly, total RNAs were purified from leukocytes in CML patients and healthy volunteers.   T7-based RNA amplification was carried out to obtain

adequate RNA for microarray experiments.   Aliquots of amplified RNA from CML patients and healthy volunteers were labeled by reverse transcription with Cy5-dCTP and Cy3-dCTP, respectively (Amersham Biosciences, Buckinghamshire, UK).   Hybridization, washing, and detection were carried out as described previously (Kaneta *et al.*, 2002 Jpn. J.

5   Cancer Res., 93, 849-856.).   Subsequently, among the up-regulated genes, the gene assigined in-house identification number C6135 was seledted.   C6135 which has the expression ratio of C6135 was greater than 5.0 in more than 60% of informative CML cases.

10   **Northern-blot analysis**

Human multiple-tissue Northern blots (Clontech, Palo Alto, CA) were hybridized with a $[\alpha\ ^{32}P]$ dCTP-labeled PCR product of C6135, a gene on the microarray.   The PCR product was prepared by RT-PCR using primers: 5'-GTGCTCTTCCTCTTCACCTTTG-3' (SEQ.ID:NO.1) and 5'-GGTGGTCGTCAAGAAACAAGTTA-3' (SEQ.ID:NO.2).

15   Pre-hybridization, hybridization and washing were performed according to the supplier's recommendations.   The blots were autoradiographed with intensifying screens at -80°C for 9 days.

Semi-quantitative RT-PCR analysis

20   Total RNA was extracted from cultured cells and clinical samples using TRIzol Reagent (Invitrogen) according to the manufacturer's protocol.   Extracted RNA was treated with DNase I (Roche) and reversely transcribed for single-stranded cDNAs using oligo(dT)$_{16}$ primer with Superscript II reverse transcriptase (Roche).   For subsequent PCR amplification by monitoring the β-actin *(ACTB)* as a quantitative control, appropriate

25   dilutions of each single-stranded cDNA were prepared.   The primer sequences were 5'-CATCCACGAAACTACCTTCAACT-3' (SEQ.ID:NO.3) and 5'-TCTCCTTAGAGAGAAGTGGGGTG-3' (SEQ.ID:NO.4) for *ACTB*; 5'-GTGCTCTTCCTCTTCACCTTTG-3' (SEQ.ID:NO.5) and 5'-GGTGGTCGTCAAGAAACAAGTTA-3' (SEQ.ID:NO.6) for C6135;

30   5'-GACAACTCACTCAAGATTGTCAG-3' (SEQ.ID:NO.7) and 5'-GATCCACGACGGACACATTG-3' *(SEQ.ID.NO.8)* for *GAPDH*.   All reactions involved initial denaturation at 94°C for 2 min followed by 21 cycles (for *ACTB and GAPDH*) or 30 cycles (for C6135) at 94°C for 30 s, 58°C for 30 s, and 72°C for 1 min, on a GeneAmp PCR system 9700 (PE Applied Biosystems).

35

Construction of expression vector

The entire coding sequence of C6135 cDNA was amplified by RT-PCR with

primers C6135-forward (5'-CGGAATTCCGATGAGTGAGGCCCGCAGG-3' (SEQ.ID: NO.9)) and C6135-reverse (5'-GGGGTACCCCAGTGGAGCTGAGCGTCCAG-3' (SE Q.ID:NO.10)). The product was inserted into the EcoRI and KpnI sites of pcDNA 3.1(-).myc.his (invitrogen), which carriers a cytomegalovirus (CMV) promoter and a

5   gene conferring neomycin resistance (pcDNA3.1(-)-C6135-myc-his). Constructs wer e confirmed by DNA sequencing.

Immunocytochemical staining

NIH3T3 cells were transfected transiently with pcDNA3.1(-)-C6135-myc.his using

10   FuGENE 6 (Roche) according to manufacture's instruction, and then were fixed with 4% paraformaldehyde, and permeablilized with 0.2% Triton X-100in PBS for 3min at room temperature. Next, the cells were covered with blocking solution (3% BSA/PBS containing 0.2% Triton X-100) for 30min at room temperature, and incubated with a rabbit anti-myc antibody (Santa Cruz Biotechnology) or a mouse monoclonal anti-Golgi 58K

15   protein (Sigma) in blocking solution for 60 min at room temperature. After washing with PBS, cells were stained by a FITC-conjugated anti-rabbit secondary antibody (Organon teknika), Rhodamine-conjugated anti-mouse secondary antibody (ICN Biomedicals) and 4', 6'-diamidine-2'- phenyl -indolendihydrochrolide (DAPI) (Roche) for 60 min at room temperature.and visualized with an Nikon Eclips E800 fluorescence microscope (Nikon,

20   Tokyo, Japan).

Growth assay

NIH3T3 cells stably expressing C6135 (NIH3T3-C6135 cells) were established by transfecting NIH3T3 cells with pcDNA3.1(-)-C6135-myc.his plasmid using FuGENE 6.

25   As a control, cells transfected with empty vector (NIH3T3-vector cells) were subcloned as well. NIH3T3-C6135 and NIH3T3-vector cells were seeded on 6-well plate (1 X 10^4 cells/well) and cell proliferation was determined by MTT assay using cell counting kit-8 (Wako pure chemicals industries) according to manufacture's instruction.

30   **Effect of antisense S-oligodeoxynucleotides on cell growth**

K562 cells plated onto 24-well plate (2X10^6 cells/well) were transfected with synthetic S-oligonucleotides (10mM) corresponding to C6135 and maintained in media containing 10% fetal bovine serum for 48 hours. MTT (3-(4,5- dimethyl- thiazol -2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assay was performed in triplicate as

35   described elsewhere (Akashi et al., 2000. Int. J. Cancer, 88, 873-880.). Sequences of the S-oligonucleotides were as follows:

antisense (5'-CTGTGTGATGGACGTCTG-3' (SEQ.ID:NO.11)),

reverse (5'-GTCTGCAGGTAGTGTGTC-3' (SEQ.ID:NO.12)).

Effect on RNAi on cell growth

The siRNA expression vector (psiH1BX) was used for RNAi. The H1 promoter
5   was cloned into the upstream of the gene specific sequence (19nt sequence from the target
transcript separated by a short spacer from the reverse complement of the same sequence)
and five thymidines as termination signal, furthermore neo cassette was integrated to
became resistance for Geneticin (Sigma). The target sequences for *RHBDF1* and *EGFP*
are 5'-GTACGTGCAGCAGGAGAAC-3' (SEQ.ID:NO.13) and
10   5'-GAAGCAGCACGACTTCTTC-3' (SEQ.ID:NO.14), respectively. The human lung
adenocarcinoma cell lines A549, H522 and LC319 were plated onto 10-cm dishes (5 X 10^5
cells/dish) and transfected with psiH1BX, psiH1BX containing EGFP target sequence
(psiH1BX-EGFP) and psiH1BX containing *RHBDF1* target sequence
(psiH1BX-*RHBDF1*) using Lipofectamine 2000 (Invitrogen) according to manufacture's
15   instruction. Cells were selected by 500 □g/ml Geneticin for one week and stained by
Giemsa solution and performed MTT assay.

Result

Identification of *RHBDF1* as an up-regulated gene in CML cells
20   Gene-expression profiles of cancer cells from 27 CML patients have been analyzed,
using a cDNA microarray representing 23,040 human genes (Kaneta *et al.*, 2002.Jpn. J.
Cancer Res., 93, 849-856.) and identified 150 genes that were commonly up-regulated in
CML cells. Among them, one gene assigned in-house code C6135 was focused on, that
was markedly up-regulated in more than 60 % of CML patients (Figure 1a). C6135
25   cDNA consisted of 2958 nucleotides (SEQ.ID.NO.15) with open reading frame 2568bp,
encoding a deduced 855 amino–acid protein (DNA sequence is available from GenBank,
accession number NM_022450) (SEQ.ID.NO.16). A homology search of the predicted
amino-acid sequence with proteins in the NCBI database (National Center for
Biotechnology Information, http://www.ncbi.nlm.nih.gov/) using the BLAST program
30   revealed some degree of homology (39% amino acid identity) of this protein to the
Rhomboid-5 of *Drosophila melanogaster*. The SMART program predicted that C6135
protein would contain a rhomboid domain consisting of the seven transmembrane domains
at the C-terminal portion and suggested its Golgi location. Comparison of C6135 protein
with *Drosophila* rhomboid family also indicated the high conservation of the rhomboid
35   domain in the family members (Figure 1b). We then termed this gene as *RHBDF1*,
Rhomboid family 1 (*Drosophila*) for reason described above. As shown in Figure 1c, a

phylogenetic tree derived from these sequences also represents that *RHBDF1* is most closely homologous to *Drosophila* Rhomboid-5.

Northern blot analysis using the *RHBDF1* cDNA clone as a probe (Figure 2a) identified a 3.1-kb transcript expressed ubiquitously but most abundantly in trachea,

5 thyroid, spinal cord, prostate, skeletal muscle, and placenta.   To further investigate the subcellular localization of *RHBDF1* protein, a plasmid expressing *RHBDF1* protein (pcDNA3.1(-) -C6135-myc-his)   was ransfected into NIH3T3 cell and performed immunocytochemical staining.   As shown in Figure 2b, *RHBDF1* protein was observed at Golgi with an anti-myc antibody.

10

Effect of *RHBDF1* on growth of NIH3T3 cells

To elucidate a potential tumorigenic role of *RHBDF1*, NIH3T3-*RHBDF1* cells were established. The NIH3T3-*RHBDF1* stably overexpressed *RHBDF1* by transfecting pcDNA3.1(-)-*RHBDF1*-myc-his into NIH3T3 cells, and confirmed the stable expression in

15 some transformants by semi-quantitative RT-PCR (Figure 3a).   Then, to investigate the growth effect of *RHBDF1* expression using these transformed clones, their growth was compared with control cells transfected with mock (NIH3T3-mock cells) by MTT assay. As shown in Figure 3b, NIH3T3-*RHBDF1* cells (#1, #2, and #3) grew at a markedly increased rate compared with control cells.   These results were confirmed in three

20 independent experiments in triplicate wells.   This finding indicates that *RHBDF1*-overexpressing NIH3T3 cells possess a growth advantage.

Growth-inhibitiory effect of antisense S-oligonucleotides and small interfering RNA (siRNA) designed to reduce expression of *RHBDF1*

25 To further assess the growth-promoting role of *RHBDF1*, five antisense S-oligonucleotides corresponding to parts of *RHBDF1* sequences were synthesized, and transfected them into K562 cells, which had shown overexpression *of RHBDF1*. Forty-eight hours after transfection, mRNA was extracted and then examined expression levels of *RHBDF1* by semi-quantitative RT-PCR.   Among the five antisense

30 S-oligonucleotides examined, one (*RHBDF1*-AS1) significantly suppressed the expression of *RHBDF1* compared with control S-oligonucleotide (*RHBDF1*-R1) having the reverse sequence of the antisense-oligonucleotide (Figure 4a).   This growth suppressive effect by *RHBDF*-AS1 was confirmed using MTT assay and confirmed that introduction of *RHBDF*-AS1 clearly suppressed growth of K562 cells compared with *RHBDF*-R1 (Figure

35 4b).

To further confirm the growth-promoting role of *RHBDF1* in K562 CML cells, the expression of endogenous *RHBDF1* gene was knocked down by mammalian vector-based

RNA interfering (RNAi) technique (see Materials and Methods) (Figure 4c).   The
transfection of the psiH1BX-*RHBDF1* resulted in reduction of expression and resulted in
growth suppression in concordant with the result using antisense S-oligonucleotide (Figure
4d).   Taken together, our finding implies that *RHBDF1* has an oncogenic function in CML
5   cells.

Recent our expression profiles revealed that *RHBDF1* was also significantly
up-regulated in acute myeloid leukemias (AML) and also lung adenocarcinomas compared
with each normal control.   Subsequent semi-quantitative RT-PCR experiments identified
the increased expression of *RHBDF1* in more than half of 14 AML samples and all of
10   seven lung adenocarcinoma samples (Figure 5a and 5b).   Therefore, to investigate the role
of *RHBDF1* in pulmonary carcinogenesis, the expression of *RHBDF1* was knocked down
in A549, LC319 and H522, lung adenocarcinoma cell lines by mammalian vector-based
RNAi and examined its effect on cell growth.   As shown in Figure 6a, 6c and 6e,
introduction of psiH1BX-*RHBDF1* clearly reduced the expression of *RHBDF1* in all of
15   lung adenocarcinoma cell lines and resulted in growth suppression of these cells while no
effect was observed in cells transfected with the control plasmids, psiH1BX and
psiH1BX-GFP siRNA expression vectors.   To further confirm the gene-specific growth
reduction by psiH1BX-*RHBDF1*, colony formation assay was performed using three lung
adenocarcinoma cell lines.   As shown in Figure 6b, 6d and 6f, introduction of
20   psiH1BX-*RHBDF1* in the three cell lines resulted in significant suppression of cell growth.
Moreover, the results of the MTT assay also showed the growth inhibitory effects when the
RHBDF1 expression was repressed (Figure 6g).   These results were verified by three
independent experiments.

25   **Discussion**
To comprehensively investigate the detailed molecular mechanism of carcinogenesis, we
have been attempting to obtain the genome-wide expression profiles of cancer cells from
CMLs, AMLs and lung adenocarcinomas by means of cDNA microarray representing
23,040 transcripts (Kaneta *et al.*, 2002 *Jpn. J. Cancer Res.*, *93, 849-856.;* Okutsu *et al.*,
30   2002. *Mol. Cancer Ther.*, 1, 1035-1042.; Kikuchi *et al.*, 2003. *Oncogene*, 22, 2192-2205.).
Among the genes up-regulated in these cancers, we identified the *RHBDF1* gene, similar to
Drosophila Rhomboid-5, that is likely to belong to the Rhomboid family.   The Rhomboid
family was isolated recently and their functions are indicated in only a limited number of
organisms and contexts.   Among them, *Drosophila* Rhomboid-1 has been identified as an
35   intramembrane serine protease that is responsible for initiating *Drosophila* epidermal
growth factor receptor (EGFR) signaling (Lee *et al.*, 2001 Cell, 107, 161-171; Urban *et al.*,
2002. *EMBO J.*, 21, 4277-4286.; Urban *et al.*, 2001. *Cell*, 107, 173-182.).   Activation of

this pathway in *Drosophila* is regulated by the selective proteolytic activation of the three transmembrane EGFR ligand precursors, Spitz, Keren and Gurken.   In their transmembrane forms, these ligands are inactive, being confined to the endoplasmic reticulum (ER).   In the signal-positive cell, Star, type2 membrane protein, exports these

5   ligands from the endoplasmic reticulum to the Golgi apparatus, where they are cleaved by rhomboid intramembrane serine proteases.   This cleavage releases the EGF ligand domains for subsequent secretion as active signals for other cells.   The protease active site of Rhomboids lies within the membrane bilayer, and the activating cleavage occurs within the ligand transmembrane domain. This proteolytic cleavage system is in contrast to other

10   known growth factors, which use cell surface metalloproteases to release the active growth factor domain   (Urban *et al.*, 2002. *Curr. Biol.*, 12, 1507-1512.).   Little is known about the function of nearly 100 currently known rhomboid-related genes that are conserved throughout evolution, but recent studies indicated that a Rhomboid from pathogenic bacterium was involved in the production of a quorum-sensing factor (Rather *et al.*, 1994.

15   *J. Bacteriol.*, 176, 5140-5144.; Gallio *et al.*, 2000. *Curr. Biol.*, 10, R693-694.), suggesting conservation of a Rhomboid-associated intercellular signaling mechanism during evolutional steps.

According to recent functional analysis of prokaryotic rhomboids as mentioned above, it has been understood that all Rhomboid proteins possess an intramembrane serine protease

20   function.   For example, *Drosophila* Rhomboids 1-4 have similar proteolytic activities and all membrane-tethered ligands are substrates for the Rhomboid proteases (Lee *et al.*, 2001.). However, although *RHBDF1* contained highly conserved rhomboid domain (Figure 1b and 1c), the essential residues for a serine protease that catalyze proteolysis were not conserved within this rhomboid domain.   Therefore, it would be of great interest to investigate

25   whether *RHBDF1* protein might have proteolytic activity against membrane-tethered EGF receptor ligands such as Spitz.   Additional direct biochemical analysis of purified *RHBDF1* protein activity will be required to answer the above questions.

Our results strongly suggested the activated *RHBDF1* to function as oncogene on the basis of the facts that stable *RHBDF1* expression enhanced cell growth, and that reduction

30   of *RHBDF1* expression by antisense S-oligonucleotide or RNAi suppressed growth of CML and lung-adenocarcinoma cells.   Furthermore, immunocytochemical staining indicated *RHBDF1* localized at Golgi apparatus like other Rhomboid proteins.   These findings suggested that *RHBDF1* might have its own target substrates that mediate *RHBDF1*-dependent signaling, although such target molecules are currently unclear.   If so,

35   identification of substrate for *RHBDF1* might provide us new clues to design novel anti-cancer drugs.

In conclusion, this study demonstrated a possible involvement of Rhomboid protein

in carcinogenesis. Since the expression of *RHBDF1* transcript is relatively low in normal human adult tissues, *RHBDF1* itself might serve as a novel therapeutic target for cancer.

Industrial Applicability

5   The expression of novel human genes *RHBDF1* is markedly elevated in CML and AML compared to normal peripheral blood cell, and lung adenocarcinoma compared to normal lung cell. Accordingly, the genes may serve as a diagnostic marker of cancer and the proteins encoded thereby may be used in diagnostic assays of cancer.

      The present inventors have also shown that the expression of novel protein

10   *RHBDF1* promotes cell growth whereas cell growth is suppressed by antisense oligonucleotides or small interfering RNAs corresponding to the *RHBDF1* gene. These findings suggest that each of *RHBDF1* proteins stimulate oncogenic activity. Thus, each of these novel oncoproteins is useful targets for the development of anti-cancer pharmaceuticals. For example, agents that block the expression of *RHBDF1*, or prevent

15   its activity may find therapeutic utility as anti-cancer agents, particularly anti-cancer agents for the treatment of CML, AML, and lung adenocarcinoma. Examples of such agents include antisense oligonucleotides, small interfering RNAs, and antibodies that recognize *RHBDF1*.

      While the invention has been described in detail and with reference to specific

20   embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope of the invention.

CLAIMS

1. An substantially pure polypeptide selected from the group consisting of:

    (a) a polypeptide comprising the amino acid sequence of SEQ ID NO: 16;

    (b) a polypeptide that comprises the amino acid sequence of SEQ ID NO: 16 in which one or more amino acids are substituted, deleted, inserted, and/or added and that has a biological activity equivalent to a protein consisting of the amino acid sequence of SEQ ID NO: 16; and

    (c) a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide consisting of the nucleotide sequence of SEQ ID NO: 15, wherein the polypeptide has a biological activity equivalent to a polypeptide consisting of the amino acid sequence of any one of SEQ ID NO: 16

2. An isolated polynucleotide encoding the polypeptide of claim 1.

3. A vector comprising the polynucleotide of claim 2.

4. A host cell harboring the polynucleotide of claim 2 or the vector of claim 3.

5. A method for producing the polypeptide of claim 1, said method comprising the steps of:

    (a) culturing the host cell of claim 4;

    (b) allowing the host cell to express the polypeptide; and

    (c) collecting the expressed polypeptide.

6. An antibody binding to the polypeptide of claim 1.

7. A polynucleotide that is complementary to the polynucleotide of claim 2 or to the complementary strand thereof and that comprises at least 15 nucleotides.

8. An antisense polynucleotide or small interfering RNA against the polynucleotide of claim 2.

9. The antisense polynucleotide of claim 8, wherein the nucleotide sequence thereof comprises the nucleotide sequence of SEQ ID NO: 11.

10. The small interfering RNA of claim 8, wherein the sense strand thereof comprises the nucleotide sequence of SEQ ID NO: 13.

11. A method for diagnosing a cell proliferative disease, said method comprising the steps of:

    (a) detecting the expression level of the gene encoding the amino acid sequence of SEQ ID NO: 16 in a biological sample of specimen; and

    (b) relating an elevation of the expression level to the disease.

12. The method of claim 11, wherein the expression level is detected by any one of the method select from the group consisting of:

    (a) detecting the mRNA encoding the amino acid sequence of SEQ ID NO: 16,

(b) detecting the protein comprising the amino acid sequence of SEQ ID NO: 16, and

(c) detecting the biological activity of the protein comprising the amino acid sequence of SEQ ID NO: 16

13. A method of screening for a compound for treating a cell proliferative disease, said method comprising the steps of:

(a) contacting a test compound with a polypeptide selected from the group consisting of:

(1) a polypeptide comprising the amino acid sequence of SEQ ID NO: 16;

(2) a polypeptide that comprises the amino acid sequence of SEQ ID NO: 16 in which one or more amino acids are substituted, deleted, inserted, and/or added and that has a biological activity equivalent to a protein consisting of the amino acid sequence of SEQ ID NO: 16; and

(3) a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide consisting of the nucleotide sequence of SEQ ID NO: 15, wherein the polypeptide has a biological activity equivalent to a polypeptide consisting of the amino acid sequence of SEQ ID NO: 16;

(b) detecting the binding activity between the polypeptide and the test compound; and

(c) selecting a compound that binds to the polypeptide.

14. A method of screening for a compound for treating a cell proliferative disease, said method comprising the steps of:

(a) contacting a candidate compound with a cell expressing a polynucleotide comprising the nucleotide sequence of SEQ ID NO: 15; and

(b) selecting a compound that reduces the expression level of the polynucleotide comparison with the expression level detected in the absence of the test compound.

15. A method of screening for a compound for treating a cell proliferative disease, said method comprising the steps of:

(a) contacting a test compound with a polypeptide selected from the group consisting of:

(1) a polypeptide comprising the amino acid sequence of SEQ ID NO: 16;

(2) a polypeptide that comprises the amino acid sequence of SEQ ID NO: 16 in which one or more amino acids are substituted, deleted, inserted, and/or added and that has a biological activity equivalent to a protein consisting of the amino acid sequence of SEQ ID NO: 16; and

(3) a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide consisting of the nucleotide sequence of SEQ ID

NO: 15, wherein the polypeptide has a biological activity equivalent to a polypeptide consisting of the amino acid sequence of SEQ ID NO: 16;

(b) detecting the biological activity of the polypeptide of step (a); and

(c) selecting a compound that suppresses the biological activity of the polypeptide in comparison with the biological activity detected in the absence of the test compound.

16. The method of claim 15, wherein the biological activity is cell-proliferating activity.

17. A method of screening for compound for treating cell proliferative disease, said method comprising the steps of:

a)   contacting a candidate compound with a cell into which a vector comprising the transcriptional regulatory region of a marker gene and a reporter gene that is expressed under the control of the transcriptional regulatory region has been introduced, wherein the   marker genes comprising nucleotide sequence of SEQ ID:NO 15

b)   measuring the activity of said reporter gene; and

c)   selecting a compound that reduces the expression level of said reporter gene in comparison with the expression level of said reporter gene detected in the absence of the test compound.

18. A method of any one of claim 11 to 17, wherein the cell-proliferative disease is cancer.

19. A composition for treating a cell proliferative disease, said composition comprising a pharmaceutically effective amount of an antisense polynucleotide or small interfering RNA against a polynucleotide encoding a polypeptide selected from the group consisting of:

(a) a polypeptide that comprises the amino acid sequence of SEQ ID NO: 16;

(b) a polypeptide that comprises the amino acid sequence of SEQ ID NO: 16 in which one or more amino acids are substituted, deleted, inserted, and/or added and that has a biological activity equivalent to a protein consisting of the amino acid sequence of SEQ ID NO: 16; and

(c) a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide consisting of the nucleotide sequence of SEQ ID NO: 15, wherein the polypeptide has a biological activity equivalent to a polypeptide consisting of the amino acid sequence of SEQ ID NO: 16

as an active ingredient, and a pharmaceutically acceptable carrier.

20. A composition for treating a cell proliferative disease, said composition comprising a pharmaceutically effective amount of an antibody against a polypeptide selected from the group consisting of:

(a) a polypeptide that comprises the amino acid sequence of SEQ ID NO: 16;

(b) a polypeptide that comprises the amino acid sequence of SEQ ID NO: 16 in which one or more amino acids are substituted, deleted, inserted, and/or added and that has a biological activity equivalent to a protein consisting of the amino acid sequence of SEQ ID NO: 16; and

(c) a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide consisting of the nucleotide sequence of SEQ ID NO: 15, wherein the polypeptide has a biological activity equivalent to a polypeptide consisting of the amino acid sequence of SEQ ID NO: 16 as an active ingredient, and a pharmaceutically acceptable carrier.

21. A composition for treating a cell proliferative disease, said composition comprising a pharmaceutically effective amount of the compound selected by the method of any one of claims 13 to 17 as an active ingredient, and a pharmaceutically acceptable carrier.

22. The composition of any one of claims 19 to 21, wherein the cell proliferative disease is cancer.

23. A method for treating a cell proliferative disease, said method comprising the step of administering a pharmaceutically effective amount of an antisense polynucleotide or small interfering RNA against a polynucleotide encoding a polypeptide selected from the group consisting of:

(1) a polypeptide comprising the amino acid sequence of SEQ ID NO: 16;

(2) a polypeptide that comprises the amino acid sequence of SEQ ID NO: 16 in which one or more amino acids are substituted, deleted, inserted, and/or added and that has a biological activity equivalent to a protein consisting of the amino acid sequence of SEQ ID NO: 16; and

(3) a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide consisting of the nucleotide sequence of SEQ ID NO: 15, wherein the polypeptide has a biological activity equivalent to a polypeptide consisting of the amino acid sequence of SEQ ID NO: 16.

24. A method for treating a cell proliferative disease, said method comprising the step of administering a pharmaceutically effective amount of an antibody against a polypeptide selected from the group consisting of:

(a) a polypeptide that comprises the amino acid sequence of SEQ ID NO: 16;

(b) a polypeptide that comprises the amino acid sequence of SEQ ID NO: 16 in which one or more amino acids are substituted, deleted, inserted, and/or added and that has a biological activity equivalent to a protein consisting of the amino acid sequence of SEQ ID NO: 16; and

(c) a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide consisting of the nucleotide sequence of SEQ ID NO: 15, wherein the polypeptide has a biological activity equivalent to a polypeptide consisting of the amino acid sequence of SEQ ID NO: 16.

25. A method for treating a cell proliferative disease, said method comprising the step of administering a pharmaceutically effective amount of a compound selected by the method of any one of claims 13 to 15.

26. The method of any one of claims 23 to 25, wherein the cell proliferative disease is cancer.

27. A method for treating or preventing a cancer, said method comprising the step of administering a pharmaceutically effective amount of a polypeptide selected from the group consisting of (a)-(c), or a polynucleotide encoding the polypeptide:

(a) a polypeptide comprising the amino acid sequence of SEQ ID NO: 16 or fragment thereof;

(b) a polypeptide that comprises the amino acid sequence of SEQ ID NO: 16 in which one or more amino acids are substituted, deleted , inserted, and/or added and that has a biological activity equivalent to a protein consisting of the amino acid sequence of SEQ ID NO: 16, or fragment thereof;

(c) a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide consisting of the nucleotide sequence of SEQ ID NO: 15, wherein the polypeptide has a biological activity equivalent to a polypeptide consisting of the amino acid sequence of SEQ ID NO: 16, or fragment thereof.

28. A method for inducing an anti tumor immunity, said method comprising the step of contacting a polypeptide selected from the group consisting of (a)-(c) with antigen presenting cells:

(a) a polypeptide comprising the amino acid sequence of SEQ ID NO: 16, or fragment thereof;

(b) a polypeptide that comprises the amino acid sequence of SEQ ID NO: 16 in which one or more amino acids are substituted, deleted, inserted, and/or added and that has a biological activity equivalent to a protein consisting of the amino acid sequence of SEQ ID NO: 16, or fragment thereof;

(c) a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide consisting of the nucleotide sequence of SEQ ID NO: 15, wherein the polypeptide has a biological activity equivalent to a polypeptide consisting of the amino acid sequence of SEQ ID NO: 16, or

fragment thereof.

29. The method for inducing an anti tumor immunity of claim 28, wherein the method further comprising the step of administering the antigen presenting cells to a subject.

30. A pharmaceutical composition for treating or preventing a cancer, said composition comprising a pharmaceutically effective amount of polypeptide selected from the group of (a)-(c), or a polynucleotide encoding the polypeptide:

(a) a polypeptide comprising the amino acid sequence of SEQ ID NO: 16, or fragment thereof;

(b) a polypeptide that comprises the amino acid sequence of   SEQ ID NO: 16 in which one or more amino acids are substituted, deleted , inserted, and/or added and that has a biological activity equivalent to a protein consisting of the amino acid sequence of SEQ ID NO: 16, or fragment thereof;

(c) a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide consisting of the nucleotide sequence of SEQ ID NO: 15, wherein the polypeptide has a biological activity equivalent to a polypeptide consisting of the amino acid sequence of SEQ ID NO: 16, or fragment thereof.

FIG.1-1

(a)

(c)

FIG.1-2

(b)-1

```
RHBDF1   P-CCIGTKGRCEITSREYCDFMRGYFHEEAT----------------------------------            627
DmRho1   P-WFILVISIEIAIFAYDRYTMPAQ------------------------------------------------     126
DmRho3   P-FFIILATLLEVLVFLWVG----------------------------------------------------       116
DmRho2   PFFIILVTLVELGFFVYHSVVTG---------------------------------------------------     190
DmRho4   PPPLTMVLFSIIEIIMFLVDVIHFQDDPN---------------------------------------------
DmRho6
DmRho5   P-FFTYWINTVQVVVLILSIICYGIAPIGIGSEKTGQVLVTSLSLQTVQHVEQRNLWIG-             1304

RHBDF1   -----------------------------------------------------LCSQVHC             634
DmRho1   -----------------------------------------------------NFGLPVP             133
DmRho3   -----------------------------------------------------------AD            118
DmRho2   -----------------------------------------------------EAAPRGP             197
DmRho4   ------------------------------------------------YQDRIGESTS               205
DmRho6
DmRho5   PRNIDLVHMGAKFAACMRRDIKITEVVTKRRHERETACCIRNDDSGCVQSSQAECSIRG            1364

RHBDF1   MDDVCGLLPFLN---------------------------------------------                 646
DmRho1   IPSDSVLVYR-----------------------------------------------                 143
DmRho3   PPEDSLLVYR-----------------------------------------------                 128
DmRho2   IPSDSMFIYR-----------------------------------------------                 207
DmRho4   GPAATLFIYN-----------------------------------------------                 225
DmRho6   -MQKVLIFK------------------------------------------------                   8
DmRho5   LYPTKSISTWKKWSPSESGPGGRISGSVCGLDPKFCDAPASIAPYEWPDDITKWPICRKT           1424

RHBDF1   ------------------------------------------------------------
DmRho1   ------------------------------------------------------------
DmRho3   ------------------------------------------------------------
DmRho2   ------------------------------------------------------------
DmRho4   ------------------------------------------------------------
DmRho6   ------------------------------------------------------------
DmRho5   NSFTQRYRYKDHTSEHMVCEVIGHPCCTGLYGECRITTREYCDFIKGYFHEEASLCSQIS           1484

RHBDF1   PEVPDQFYRLWLSLFLHAGILHCLVSICFQMTVLRDLEKLAGWHRIA             693
DmRho1   PDRRLQVWRFFSYMFLHANWFHLGFNIVIQLFFGIPLEVMHGTARIG             190
DmRho3   PDQRLQLWRFLSYALLHASWLHLGYNVLTQLLFGVPLELVHGSLRTG             175
DmRho2   PDKRHEIWRFLFYMVLHAGWLHLGFNVAVQLVFGLPLEMVHGSTRIA             254
DmRho4   PYKRYEGWRFVSYMFVHVGIMHLMMNLIIQIFLGIALELVHHWWRVG             272
DmRho6   PEWNVEYWRLLTYMLLHSDYWHLSLNICFQCFIGICLEVEQGHWRLA              55
DmRho5   CLNNVCGMFPFISVETPDQLYRLLTSLCMHAGILHLAITLIFQHLFLADLERLIGTVRTA           1544
```

FIG.1-3

(b)-2

```
RHBDF1  IIYLLSGVTGNLASAIFLPYRAEVGPAGSQFGILACLFVELFQS-WQILARPWRAFFKLL  752
DmRho1  VIYMAGVFAGSLGTSVVDSEVFLVGASGGGVYALLAAHLANITLN-YAHMKSASTQLGSVV  249
DmRho3  VIYMAGVLAGSLGTSVVDSEVFLVGASGGGVYALLAAQLASLLLN-FGQMRHGVIQLMAVI  234
DmRho2  CIYFSGVLAGSLGTSIFDPDVFLVGASGGGVYALLAAHLANVLLN-YHQMRYGVIKLLHIL  313
DmRho4  LVYLAGVLAGSMGTSLTSPRIFLAGASGGGVYALITAHIATIIMN-YSEMEYAIVQLLAFL  331
DmRho6  VVYMVGGVAGSLANAWLQPHLHLMGASAGVYAMLGSHVPHLVLN-FSQLSHRFARIASLL  114
DmRho5  IVYIMSGFAGNLTSAILVPHRPEVGPSASLSGVVASLIALLVWMHWKYLHKPHIALFKLL  1604

RHBDF1  AVVLFLFTFGLLPWIDN--------------------------FAHISGFISGLFLSF  784
DmRho1  IFVSCDLGYALYTQYF-----------------------DGSAFAKGPQVSYIAHLTGAL  286
DmRho3  LFVFCDLGYALYSREL---------------AMHQLQTRPSVSYIAHMTGAL  271
DmRho2  VFVSFDFGFAIYARYAGDELQLG-------SSSEFPAIDQAETAGAVSYVAHLAGAI  363
DmRho4  VFCFTDLGTSVYRHLT-----------------DQHDQIGYVAHLSGAV  363
DmRho6  ILLLSDVGFTTYHFCHN--------------HNRNPRTSLEAHIGGGGV  148
DmRho5  LLCSVLVGIGTLPYQLNFLGLLAGVICGCLLTMSLVPFTTFSKYGRKKKINLIWTCVLFH  1664

RHBDF1  AFLPYISFGKFDLYRKRCQIIFQVVFLGLLAGLVVLFYVYPVRCEWCEFLTCIPFTDKF  844
DmRho1  AGLTIGFLVLKNFGHREYEQLIWWLALG-VYCAFTVFA-VFNLINTVTAQLMEEQ-GEVI  344
DmRho3  AGISVGLLLLRQLDGGLRPRPLRWLALG-VWCIFSAFGIAFNLVNTVTAQLLAEEEGQVI  330
DmRho2  AGLTIGLLVLKSFEQRLHEQLLWWIALG-TYLALVVFAIAFNIMNGFAMFNIRVEKIR--  420
DmRho4  AGLLVGIGVLRNLEVRRWERILWWVAVI-VYFALMTTGIIIHVFVPDYFPKQEYN----  417
DmRho6  AGILCGFIVYRRLQPANQKAIYF----------------------  171
DmRho5  VVVYTAMIVTFYIHPSEFHS-ISFVDMFS-NSNGYDNFTNADHHGVDVVSSNTRYSQTQNS  1723

RHBDF1  CEKYELDAQLH-------  855
DmRho1  TQHLLHDLGVS-------  355
DmRho3  RQHLMNDLGMG-------  341
DmRho2  VT-ETIFNDFQV-------  431
DmRho4  -------
DmRho6  -------
DmRho5  QYYYHHHSDDIIRKSVTFTEKALVSHILYPTAP  1756
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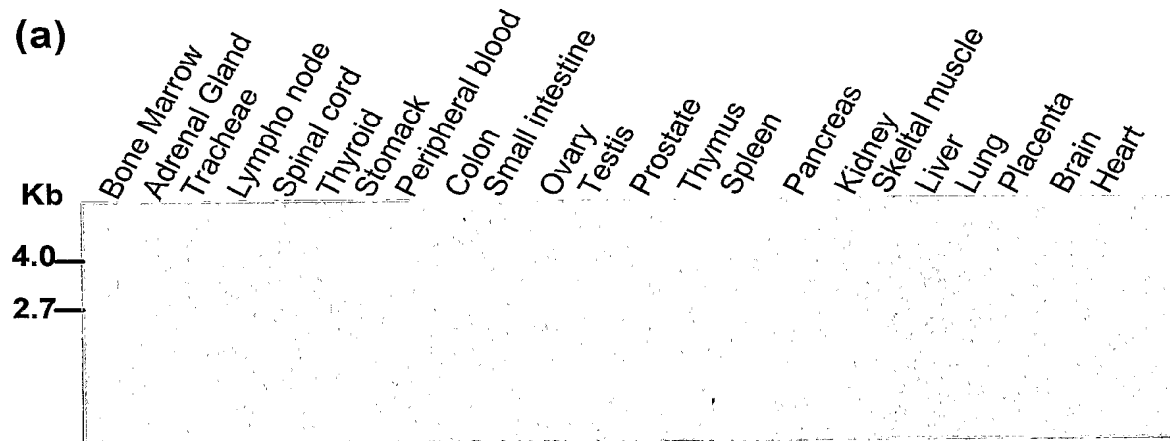
FIG. 2

(a)

(b)

Myc -tagged C6135 (FITC)   Golgi (Rhodamine)   Merge
Nuclear (DAPI)   Nuclear (DAPI)

FIG. 3

(a)

(b)

FIG. 4

FIG. 5

(a)

(b)

FIG. 6-1

(a)

RHBDF1

b-actin

psiH1BX-Mock   psiH1BX-GFP   psiH1BX-*RHBDF1*#1

(c)

RHBDF1

b-actin

(d)

psiH1BX-Mock   psiH1BX-GFP   psiH1BX-*RHBDF1*#1

FIG. 6-2

(e)

	psiH1BX -Mock	psiH1BX -GFP	psiH1BX -RHBDF1#1
RHBDF1			
β-actin			

(f)

psiH1BX-Mock   psiH1BX -GFP   psiH1BX-RHBDF1#1

FIG. 6-3

(g)

SEQUENCE LISTING

<110> ONCOTHERAPY SCIENCE, INC.

JAPAN AS REPRESENTED BY THE PRESIDENT OF THE UNIVERSITY OF TOKYO

<120> GENES AND POLYPEPTIDES RELATING TO HUMAN MYELOID LEUKEMIA

<130> ONC-A0213P2

<150> US 60/414,867

<151> 2002-09-30

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<211> 28

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<223> Artificially synthesized S-oligonucleotide sequence for Antisense

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<210> 12

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<212> DNA

<213> Artificial

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<223> Artificially synthesized S-oligonucleotides for Antisense

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gtctgcaggt agtgtgtc   18

<210> 13

<211> 19

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<400> 13

gtacgtgcag caggagaac   19

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<223> Target sequence for Si-RNA

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gaagcagcac gacttcttc   19

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cccccagag accccggcg gcggcagcct gccttgctct gccaggaacc atg agt   116
                                                         Met Ser
                                                          1

gag gcc cgc agg gac agc acg agc agc ctg cag cgc aag aag cca ccc   164
Glu Ala Arg Arg Asp Ser Thr Ser Ser Leu Gln Arg Lys Lys Pro Pro
          5   10   15

tgg cta aag ctg gac att ccc tct gcg gtg ccc ctg acg gca gaa gag   212
Trp Leu Lys Leu Asp Ile Pro Ser Ala Val Pro Leu Thr Ala Glu Glu
         20   25   30

ccc agc ttc ctg cag ccc ctg agg cga cag gct ttc ctg agg agt gtg   260
Pro Ser Phe Leu Gln Pro Leu Arg Arg Gln Ala Phe Leu Arg Ser Val
35   40   45   50

agt atg cca gcc gag aca gcc cac atc tct tca ccc cac cat gag ctc   308
Ser Met Pro Ala Glu Thr Ala His Ile Ser Ser Pro His His Glu Leu
              55   60   65

cgg cgg ccg gtg ctg caa cgc cag acg tcc atc aca cag acc atc cgc   356
Arg Arg Pro Val Leu Gln Arg Gln Thr Ser Ile Thr Gln Thr Ile Arg
              70   75   80

agg ggg acc gcc gac tgg ttt gga gtg agc aag gac agt gac agc acc   404
Arg Gly Thr Ala Asp Trp Phe Gly Val Ser Lys Asp Ser Asp Ser Thr
              85   90   95

cag aaa tgg cag cgc aag agc atc cgt cac tgc agc cag cgc tac ggg   452
Gln Lys Trp Gln Arg Lys Ser Ile Arg His Cys Ser Gln Arg Tyr Gly
100   105   110

aag ctg aag ccc cag gtc ctc cgg gag ctg gac ctg ccc agc cag gac   500
Lys Leu Lys Pro Gln Val Leu Arg Glu Leu Asp Leu Pro Ser Gln Asp
115   120   125   130

aac gtg tcg ctg acc agc acc gag acg cca ccc cca ctc tac gtg ggg   548

Asn Val Ser Leu Thr Ser Thr Glu Thr Pro Pro Pro Leu Tyr Val Gly
                    135   140   145

cca tgc cag ctg ggc atg cag aag atc ata gac ccc ctg gcc cgt ggc   596
Pro Cys Gln Leu Gly Met Gln Lys Ile Ile Asp Pro Leu Ala Arg Gly
                    150   155   160

cgt gcc ttc cgt gtg gca gat gac act gcg gaa ggc ctg agt gcc cca   644
Arg Ala Phe Arg Val Ala Asp Asp Thr Ala Glu Gly Leu Ser Ala Pro
                    165   170   175

cac act ccc gtc acg ccg ggt gct gcc tcc ctc tgc tcc ttc tcc agc   692
His Thr Pro Val Thr Pro Gly Ala Ala Ser Leu Cys Ser Phe Ser Ser
                    180   185   190

tcc cgc tca ggt ttc cac cgg ctc ccg cgg cgg cgc aag cga gag tcg   740
Ser Arg Ser Gly Phe His Arg Leu Pro Arg Arg Arg Lys Arg Glu Ser
195   200   205   210

gtg gcc aag atg agc ttc cgg gcg gcc gca gcg ctg atg aaa ggc cgc   788
Val Ala Lys Met Ser Phe Arg Ala Ala Ala Ala Leu Met Lys Gly Arg
                    215   220   225

tcc gtt agg gat ggc acc ttt cgc cgg gca cgg cgt cga agc ttc act   836
Ser Val Arg Asp Gly Thr Phe Arg Arg Ala Arg Arg Arg Ser Phe Thr
                    230   235   240

```
cca gct agc ttt ctg gag gag gac aca act gat ttc ccc gat gag ctg      884
Pro Ala Ser Phe Leu Glu Glu Asp Thr Thr Asp Phe Pro Asp Glu Leu
        245             250             255


gac aca tcc ttc ttt gcc cgg gaa ggt atc ctc cat gaa gag ctg tcc      932
Asp Thr Ser Phe Phe Ala Arg Glu Gly Ile Leu His Glu Glu Leu Ser
        260             265             270


aca tac ccg gat gaa gtt ttc gag tcc cca tcg gag gca gcg cta aag      980
Thr Tyr Pro Asp Glu Val Phe Glu Ser Pro Ser Glu Ala Ala Leu Lys
275             280             285             290


gac tgg gag aag gca ccg gag cag gcg gac ctc acc ggc ggg gcc ctg      1028
Asp Trp Glu Lys Ala Pro Glu Gln Ala Asp Leu Thr Gly Gly Ala Leu
        295             300             305


gac cgc agc gag ctt gag cgc agc cac ctg atg ctg ccc ttg gag cga      1076
Asp Arg Ser Glu Leu Glu Arg Ser His Leu Met Leu Pro Leu Glu Arg
        310             315             320


ggc tgg cgg aag cag aag gag ggc gcc gca gcc ccg cag ccc aag gtg      1124
Gly Trp Arg Lys Gln Lys Glu Gly Ala Ala Ala Pro Gln Pro Lys Val
        325             330             335


cgg ctc cga cag gag gtg gtg agc acc gcg ggg ccg cga cgg ggc cag      1172
```

Arg Leu Arg Gln Glu Val Val Ser Thr Ala Gly Pro Arg Arg Gly Gln
    340   345   350

cgt atc gcg gtg ccg gtg cgc aag ctc ttc gcc cgg gag aag cgg ccg   1220
Arg Ile Ala Val Pro Val Arg Lys Leu Phe Ala Arg Glu Lys Arg Pro
355   360   365   370

tat ggg ctg ggc atg gtg gga cgg ctc acc aac cgc acc tac cgc aag   1268
Tyr Gly Leu Gly Met Val Gly Arg Leu Thr Asn Arg Thr Tyr Arg Lys
        375   380   385

cgc atc gac agc ttc gtc aag cgc cag atc gag gac atg gac gac cac   1316
Arg Ile Asp Ser Phe Val Lys Arg Gln Ile Glu Asp Met Asp Asp His
        390   395   400

agg ccc ttc ttc acc tac tgg ctt acc ttc gtg cac tcg ctc gtc acc   1364
Arg Pro Phe Phe Thr Tyr Trp Leu Thr Phe Val His Ser Leu Val Thr
     ·405   410   415

atc cta gcc gtg tgc atc tat ggc atc gcg ccc gtg ggc ttc tcg cag   1412
Ile Leu Ala Val Cys Ile Tyr Gly Ile Ala Pro Val Gly Phe Ser Gln
        420   425   430

cat gag acg gtg gac tcg gtg ctg cgg aac cgc ggg gtc tac gag aac   1460
His Glu Thr Val Asp Ser Val Leu Arg Asn Arg Gly Val Tyr Glu Asn
        435   440   445   450

gtc aag tac gtg cag cag gag aac ttc tgg atc ggg ccc agc tcg gag   1508
Val Lys Tyr Val Gln Gln Glu Asn Phe Trp Ile Gly Pro Ser Ser Glu
            455   460   465

gcc ctc atc cac ctg ggc gcc aag ttt tcg ccc tgc atg cgc cag gac   1556
Ala Leu Ile His Leu Gly Ala Lys Phe Ser Pro Cys Met Arg Gln Asp
            470   475   480

ccg cag gtg cac agc ttc att cgc tcg gcg cgc gag cgc gag aag cac   1604
Pro Gln Val His Ser Phe Ile Arg Ser Ala Arg Glu Arg Glu Lys His
            485   490   495

tcc gcc tgc tgc gtg cgc aac gac agg tcg ggc tgc gtg cag acc tcg   1652
Ser Ala Cys Cys Val Arg Asn Asp Arg Ser Gly Cys Val Gln Thr Ser
            500   505   510

gag gag gag tgc tcg tcc acg ctg gca gtg tgg gtg aag tgg ccc atc   1700
Glu Glu Glu Cys Ser Ser Thr Leu Ala Val Trp Val Lys Trp Pro Ile
515   520   525   530

cat ccc agc gcc cca gag ctt gcg ggc cac aag aga cag ttt ggc tct   1748
His Pro Ser Ala Pro Glu Leu Ala Gly His Lys Arg Gln Phe Gly Ser
            535   540   545

gtc tgc cac cag gat ccc agg gtg tgt gat gag ccc tcc tcc gaa gac   1796

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Val Cys His Gln Asp Pro Arg Val Cys Asp Glu Pro Ser Ser Glu Asp
          550                 555                 560


cct cat gag tgg cca gaa gac atc acc aag tgg ccg atc tgc acc aaa        1844
Pro His Glu Trp Pro Glu Asp Ile Thr Lys Trp Pro Ile Cys Thr Lys
          565                 570                 575


aac agc gct ggg aac cac acc aac cat ccc cac atg gac tgt gtc atc        1892
Asn Ser Ala Gly Asn His Thr Asn His Pro His Met Asp Cys Val Ile
          580                 585                 590


aca gga cgg ccc tgc tgc att ggc acc aag ggc agg tgt gag atc acc        1940
Thr Gly Arg Pro Cys Cys Ile Gly Thr Lys Gly Arg Cys Glu Ile Thr
595                 600                 605                 610


tcc cgg gag tac tgt gac ttc atg agg ggc tac ttc cat gag gag gcc        1988
Ser Arg Glu Tyr Cys Asp Phe Met Arg Gly Tyr Phe His Glu Glu Ala
          615                 620                 625


acg ctc tgc tct cag gtg cac tgc atg gat gat gtg tgt ggg ctc ctg        2036
Thr Leu Cys Ser Gln Val His Cys Met Asp Asp Val Cys Gly Leu Leu
          630                 635                 640


cct ttt ctc aac ccc gag gtg cct gac cag ttc tac cgc ctg tgg cta        2084
Pro Phe Leu Asn Pro Glu Val Pro Asp Gln Phe Tyr Arg Leu Trp Leu
          645                 650                 655
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tcc ctc ttc ctg cac gcc ggg atc ttg cac tgc ctg gtg tcc atc tgc   2132
Ser Leu Phe Leu His Ala Gly Ile Leu His Cys Leu Val Ser Ile Cys
    660   665   670

ttc cag atg act gtc ctg cgg gac ctg gag aag ctg gca ggc tgg cac   2180
Phe Gln Met Thr Val Leu Arg Asp Leu Glu Lys Leu Ala Gly Trp His
675   680   685   690

cgc ata gcc atc atc tac ctg ctg agt ggt gtc acc ggc aac ctg gcc   2228
Arg Ile Ala Ile Ile Tyr Leu Leu Ser Gly Val Thr Gly Asn Leu Ala
        695   700   705

agt gcc atc ttc ctg cca tac cga gca gag gtg ggt cct gct ggc tcc   2276
Ser Ala Ile Phe Leu Pro Tyr Arg Ala Glu Val Gly Pro Ala Gly Ser
        710   715   720

cag ttc ggc atc ctg gcc tgc ctc ttc gtg gag ctc ttc cag agc tgg   2324
Gln Phe Gly Ile Leu Ala Cys Leu Phe Val Glu Leu Phe Gln Ser Trp
        725   730   735

cag atc ctg gcg cgg ccc tgg cgt gcc ttc ttc aag ctg ctg gct gtg   2372
Gln Ile Leu Ala Arg Pro Trp Arg Ala Phe Phe Lys Leu Leu Ala Val
        740   745   750

gtg ctc ttc ctc ttc acc ttt ggg ctg ctg ccg tgg att gac aac ttt   2420

Val Leu Phe Leu Phe Thr Phe Gly Leu Leu Pro Trp Ile Asp Asn Phe

755   760   765   770

gcc cac atc tcg ggg ttc atc agt ggc ctc ttc ctc tcc ttc gcc ttc   2468

Ala His Ile Ser Gly Phe Ile Ser Gly Leu Phe Leu Ser Phe Ala Phe

                   775   780   785

ttg ccc tac atc agc ttt ggc aag ttc gac ctg tac cgg aaa cgc tgc   2516

Leu Pro Tyr Ile Ser Phe Gly Lys Phe Asp Leu Tyr Arg Lys Arg Cys

              790   795   800

cag atc atc atc ttt cag gtg gtc ttc ctg ggc ctc ctg gct ggc ctg   2564

Gln Ile Ile Ile Phe Gln Val Val Phe Leu Gly Leu Leu Ala Gly Leu

              805   810   815

gtg gtc ctc ttc tac gtc tat cct gtc cgc tgt gag tgg tgt gag ttc   2612

Val Val Leu Phe Tyr Val Tyr Pro Val Arg Cys Glu Trp Cys Glu Phe

              820   825   830

ctc acc tgc atc ccc ttc act gac aag ttc tgt gag aag tac gaa ctg   2660

Leu Thr Cys Ile Pro Phe Thr Asp Lys Phe Cys Glu Lys Tyr Glu Leu

835   840   845   850

gac gct cag ctc cac tga gctggctgcg ggctccagcg gccgtgtgct   2708

Asp Ala Gln Leu His

              855

ccagcaggcc agagccagac acgacctccc tgagcctcac aggcttacag gagtcacctg   2768

ctccatgtgg ggactggcct gtttcctgaa cacagacctc tttcttgtgc cttgttcact   2828

tctgttgaac ccctcgtact gccgggcatt tattatacta cttcctgtca taaccttcta   2888

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<400> 16

Met Ser Glu Ala Arg Arg Asp Ser Thr Ser Ser Leu Gln Arg Lys Lys
1   5   10   15

Pro Pro Trp Leu Lys Leu Asp Ile Pro Ser Ala Val Pro Leu Thr Ala
            20   25   30

Glu Glu Pro Ser Phe Leu Gln Pro Leu Arg Arg Gln Ala Phe Leu Arg
        35   40   45

Ser Val Ser Met Pro Ala Glu Thr Ala His Ile Ser Ser Pro His His
    50   55   60

Glu Leu Arg Arg Pro Val Leu Gln Arg Gln Thr Ser Ile Thr Gln Thr
65   70   75   80

Ile Arg Arg Gly Thr Ala Asp Trp Phe Gly Val Ser Lys Asp Ser Asp
                    85   90   95

Ser Thr Gln Lys Trp Gln Arg Lys Ser Ile Arg His Cys Ser Gln Arg
                    100   105   110

Tyr Gly Lys Leu Lys Pro Gln Val Leu Arg Glu Leu Asp Leu Pro Ser
                115   120   125

Gln Asp Asn Val Ser Leu Thr Ser Thr Glu Thr Pro Pro Pro Leu Tyr
    130   135   140

Val Gly Pro Cys Gln Leu Gly Met Gln Lys Ile Ile Asp Pro Leu Ala
145   150   155   160

Arg Gly Arg Ala Phe Arg Val Ala Asp Asp Thr Ala Glu Gly Leu Ser
                165   170   175

Ala Pro His Thr Pro Val Thr Pro Gly Ala Ala Ser Leu Cys Ser Phe

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                 180                    185                    190


Ser Ser Ser Arg Ser Gly Phe His Arg Leu Pro Arg Arg Arg Lys Arg
             195                    200                    205


Glu Ser Val Ala Lys Met Ser Phe Arg Ala Ala Ala Ala Leu Met Lys
         210                    215                    220


Gly Arg Ser Val Arg Asp Gly Thr Phe Arg Arg Ala Arg Arg Arg Ser
225                    230                    235                    240


Phe Thr Pro Ala Ser Phe Leu Glu Glu Asp Thr Thr Asp Phe Pro Asp
                 245                    250                    255


Glu Leu Asp Thr Ser Phe Phe Ala Arg Glu Gly Ile Leu His Glu Glu
                 260                    265                    270


Leu Ser Thr Tyr Pro Asp Glu Val Phe Glu Ser Pro Ser Glu Ala Ala
             275                    280                    285


Leu Lys Asp Trp Glu Lys Ala Pro Glu Gln Ala Asp Leu Thr Gly Gly
         290                    295                    300


Ala Leu Asp Arg Ser Glu Leu Glu Arg Ser His Leu Met Leu Pro Leu
305                    310                    315                    320
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Glu Arg Gly Trp Arg Lys Gln Lys Glu Gly Ala Ala Ala Pro Gln Pro
       325   330   335

Lys Val Arg Leu Arg Gln Glu Val Val Ser Thr Ala Gly Pro Arg Arg
       340   345   350

Gly Gln Arg Ile Ala Val Pro Val Arg Lys Leu Phe Ala Arg Glu Lys
       355   360   365

Arg Pro Tyr Gly Leu Gly Met Val Gly Arg Leu Thr Asn Arg Thr Tyr
       370   375   380

Arg Lys Arg Ile Asp Ser Phe Val Lys Arg Gln Ile Glu Asp Met Asp
385   390   395   400

Asp His Arg Pro Phe Phe Thr Tyr Trp Leu Thr Phe Val His Ser Leu
       405   410   415

Val Thr Ile Leu Ala Val Cys Ile Tyr Gly Ile Ala Pro Val Gly Phe
       420   425   430

Ser Gln His Glu Thr Val Asp Ser Val Leu Arg Asn Arg Gly Val Tyr
       435   440   445

Glu Asn Val Lys Tyr Val Gln Gln Glu Asn Phe Trp Ile Gly Pro Ser
       450   455   460

Ser Glu Ala Leu Ile His Leu Gly Ala Lys Phe Ser Pro Cys Met Arg

465   470   475   480

Gln Asp Pro Gln Val His Ser Phe Ile Arg Ser Ala Arg Glu Arg Glu

                    485   490   495

Lys His Ser Ala Cys Cys Val Arg Asn Asp Arg Ser Gly Cys Val Gln

                    500   505   510

Thr Ser Glu Glu Glu Cys Ser Ser Thr Leu Ala Val Trp Val Lys Trp

               515   520   525

Pro Ile His Pro Ser Ala Pro Glu Leu Ala Gly His Lys Arg Gln Phe

           530   535   540

Gly Ser Val Cys His Gln Asp Pro Arg Val Cys Asp Glu Pro Ser Ser

545   550   555   560

Glu Asp Pro His Glu Trp Pro Glu Asp Ile Thr Lys Trp Pro Ile Cys

                    565   570   575

Thr Lys Asn Ser Ala Gly Asn His Thr Asn His Pro His Met Asp Cys

                    580   585   590

Val Ile Thr Gly Arg Pro Cys Cys Ile Gly Thr Lys Gly Arg Cys Glu

595   600   605

Ile Thr Ser Arg Glu Tyr Cys Asp Phe Met Arg Gly Tyr Phe His Glu
    610   615   620

Glu Ala Thr Leu Cys Ser Gln Val His Cys Met Asp Asp Val Cys Gly
625   630   635   640

Leu Leu Pro Phe Leu Asn Pro Glu Val Pro Asp Gln Phe Tyr Arg Leu
                    645   650   655

Trp Leu Ser Leu Phe Leu His Ala Gly Ile Leu His Cys Leu Val Ser
                    660   665   670

Ile Cys Phe Gln Met Thr Val Leu Arg Asp Leu Glu Lys Leu Ala Gly
            675   680   685

Trp His Arg Ile Ala Ile Ile Tyr Leu Leu Ser Gly Val Thr Gly Asn
    690   695   700

Leu Ala Ser Ala Ile Phe Leu Pro Tyr Arg Ala Glu Val Gly Pro Ala
705   710   715   720

Gly Ser Gln Phe Gly Ile Leu Ala Cys Leu Phe Val Glu Leu Phe Gln
                    725   730   735

Ser Trp Gln Ile Leu Ala Arg Pro Trp Arg Ala Phe Phe Lys Leu Leu
       740   745   750

Ala Val Val Leu Phe Leu Phe Thr Phe Gly Leu Leu Pro Trp Ile Asp
       755   760   765

Asn Phe Ala His Ile Ser Gly Phe Ile Ser Gly Leu Phe Leu Ser Phe
       770   775   780

Ala Phe Leu Pro Tyr Ile Ser Phe Gly Lys Phe Asp Leu Tyr Arg Lys
785   790   795   800

Arg Cys Gln Ile Ile Ile Phe Gln Val Val Phe Leu Gly Leu Leu Ala
          805   810   815

Gly Leu Val Val Leu Phe Tyr Val Tyr Pro Val Arg Cys Glu Trp Cys
       820   825   830

Glu Phe Leu Thr Cys Ile Pro Phe Thr Asp Lys Phe Cys Glu Lys Tyr
       835   840   845

Glu Leu Asp Ala Gln Leu His
       850   855

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7   C07K14/705   C07K16/18   C12N15/12   C12Q1/68   G01N33/50
        A61K35/00   A61K38/00   A61K39/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7   C07K   C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, MEDLINE, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE SWALL 'Online! 1 December 2001 (2001-12-01) retrieved from EBI Database accession no. Q96CC6 XP002260981	1-10
Y	abstract -& DATABASE EMBL 'Online! 23 September 2001 (2001-09-23) retrieved from EBI Database accession no. BC014425 XP002260982 abstract ---	11-30
Y	WO 02 05843 A (ROBERTSON STEPHANIE A ;BELVIN MARCIA (US); CHEN CHANGYOU (US); FRI) 24 January 2002 (2002-01-24) the whole document ---	11-30

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X	Further documents are listed in the continuation of box C.

X	Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 November 2003	27/11/2003

Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Authorized officer Rutz, B

INTERNATIONAL SEARCH REPORT

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE SWALL 'Online! 1 March 2001 (2001-03-01) retrieved from EBI Database accession no. Q9H6E1 XP002260983 abstract ---	1-10
X	DATABASE SWALL 'Online! 1 December 2001 (2001-12-01) retrieved from EBI Database accession no. Q96S34 XP002260984 abstract ---	1-10
X	DATABASE SWALL 'Online! 1 March 2002 (2002-03-01) retrieved from EBI Database accession no. Q8VIK0 XP002260985 abstract ---	1-10
A	KIELMAN M F ET AL: "Characterization and comparison of the human and mouse Dist1/alpha-globin complex reveals a tightly packed multiple gene cluster containing differentially expressed transcription units." GENOMICS. UNITED STATES 15 MAR 1996, vol. 32, no. 3, 15 March 1996 (1996-03-15), pages 341-351, XP002260977 ISSN: 0888-7543 ---	
A	LI HUIYU ET AL: "CDNA microarray analysis of chronic myeloid leukemia." INTERNATIONAL JOURNAL OF HEMATOLOGY. IRELAND MAY 2002, vol. 75, no. 4, May 2002 (2002-05), pages 388-393, XP009020831 ISSN: 0925-5710 ---	
A	KANETA YASUYUKI ET AL: "Prediction of sensitivity to STI571 among chronic myeloid leukemia patients by genome-wide cDNA microarray analysis." JAPANESE JOURNAL OF CANCER RESEARCH: GANN. JAPAN AUG 2002, vol. 93, no. 8, August 2002 (2002-08), pages 849-856, XP002260979 ISSN: 0910-5050 ---	

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INTERNATIONAL SEARCH REPORT

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	OHMINE K ET AL: "CHARACTERIZATION OF STAGE PROGRESSION IN CHRONIC MYELOID LEUKEMIA BY DNA MICROARRAY WITH PURIFIED HEMATOPOIETIC STEM CELLS" ONCOGENE, BASINGSTOKE, HANTS, GB, vol. 20, no. 57, 2001, pages 8249-8257, XP002952628 ISSN: 0950-9232	
A	KLÄMBT CHRISTIAN: "EGF receptor signalling: roles of star and rhomboid revealed" CURRENT BIOLOGY, CURRENT SCIENCE,, GB, vol. 12, no. 1, 8 January 2002 (2002-01-08), pages R21-R23, XP002248123 ISSN: 0960-9822	
P,X	WO 03 000928 A (PETERSEN MIKKEL WANDAHL ;PEDERSEN NINA (DK); MORTENSEN SHILA (DK);) 3 January 2003 (2003-01-03) the whole document -& DATABASE PATENT_PRT 'Online! 21 March 2003 (2003-03-21) retrieved from EBI Database accession no. AX658345 XP002260986 abstract	1-30
P,A	KOONIN EUGENE V ET AL: "The rhomboids: a nearly ubiquitous family of intramembrane serine proteases that probably evolved by multiple ancient horizontal gene transfers." GENOME BIOLOGY ENGLAND 2003, vol. 4, no. 3, 2003, page R19 XP002260980 ISSN: 1465-6914	

INTERNATIONAL SEARCH REPORT

Box I   Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 23-29 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:   21, 22, 25, 26 (all partially)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)

FURTHER INFORMATION CONTINUED FROM   PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.:   21, 22, 25, 26 (all partially)

Present claims 21, 22, 25 and 26 relate to a composition or a method
containing a compound defined by reference to a desirable characteristic
or property, namely compound identified by the method of any one of
claims 13-17.
The claims cover compositions and methods methods containing all
compounds having this characteristic or property, whereas the application
provides support within the meaning of Article 6 PCT and/or disclosure
within the meaning of Article 5 PCT for only a very limited number of
such compounds. In the present case, the claims so lack support, and the
application so lacks disclosure, that a meaningful search over the whole
of the claimed scope is impossible. Independent of the above reasoning,
the claims also lack clarity (Article 6 PCT). An attempt is made to
define the compoundby reference to a result to be achieved. Again, this
lack of clarity in the present case is such as to render a meaningful
search over the whole of the claimed scope impossible. Consequently, the
search has been carried out for those parts of the claims which appear to
be clear, supported and disclosed, namely those parts relating to the
antibody, antisense and RNAi molecules.

The applicant's attention is drawn to the fact that claims, or parts of
claims, relating to inventions in respect of which no international
search report has been established need not be the subject of an
international preliminary examination (Rule 66.1(e) PCT). The applicant
is advised that the EPO policy when acting as an International
Preliminary Examining Authority is normally not to carry out a
preliminary examination on matter which has not been searched. This is
the case irrespective of whether or not the claims are amended following
receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 0205843	A	24-01-2002	AU	7696701 A	30-01-2002
			CA	2416445 A1	24-01-2002
			EP	1301611 A2	16-04-2003
			WO	0205843 A2	24-01-2002
			US	2003165497 A1	04-09-2003
			US	2002022029 A1	21-02-2002
WO 03000928	A	03-01-2003	WO	03000928 A2	03-01-2003